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# PATHOLOGY OF MUSCLE CHANGES IN EXPERIMENTAL POLIOMYELITIS ENHANCED WITH AID OF CORTISONE

STANLEY M. ARONSON, M.D.
AND
GREGORY SHWARTZMAN, M.D.
NEW YORK

THE CAPACITY to propagate within striated muscle is a property shared by numerous viruses, especially those of the encephalitis group, namely, Coxsackie,[1] mouse encephalitis,[2] Col-SK,[3] vesicular stomatitis,[4] and St. Louis encephalitis.[5] Myotropism, while an inherent property of these viruses, emerges only under appropriate conditions[6] (i. e., animal species, age, and route of administration). The search for reproducible extraneural lesions in the course of experimental poliomyelitis has been consistently unfruitful.

Shwartzman demonstrated that cortisone potentiates the paralytic morbidity and mortality of the Syrian hamster infected with Type 2 poliomyelitis virus.[7] Following such enhancement of the experimental disease, visceral lesions, including myositis, appear.[8] The present report embodies the histological studies relative to the muscle lesion.

From the Department of Microbiology, Mount Sinai Hospital.

This study was aided by a grant from the National Foundation for Infantile Paralysis.

1. (*a*) Godman, G. C.; Bunting, H., and Melnick, J. L.: The Histopathology of Coxsackie Virus Infection in Mice: I. Morphologic Observations with 4 Different Viral Types, Am. J. Path. **28**:223, 1952; (*b*) II. Histochemical Observations on the Lesions in Muscle and Fat, ibid. **28**:583, 1952. (*c*) Gifford, R., and Dalldorf, G.: The Morbid Anatomy of Experimental Coxsackie Virus Infection, ibid. **27**:1047, 1951.

2. Rustigian, R., and Pappenheimer, A. M.: Myositis in Mice Following Intramuscular Injection of Viruses of the Mouse Encephalomyelitis Group and of Certain Other Neurotropic Viruses, J. Exper. Med. **89**:69, 1949.

3. Verlinde, J. D.: A Comparative Study on Muscular Involvement in Experimental Poliomyelitis Induced by Various Strains of Virus, and the Effect of Hyaluronidase on the Intramuscular Inoculation, Arch. ges. Virusforschung **4**:546, 1952.

4. Sabin, A., and Olitsky, P. K.: Influence of Host Factors on Neuroinvasiveness of Vesicular Stomatitis Virus: Effect of Age and Pathway of Infection on the Character and Localization of Lesions in the Central Nervous System, J. Exper. Med. **67**:201, 1938.

5. Peck, J. L., Jr., and Sabin, A.: Multiplication and Spread of the Virus of St. Louis Encephalitis with Special Emphasis on its Fate in the Alimentary Tract, J. Exper. Med. **85**:647, 1947.

6. Footnotes 1, 2, 3, 4 and 5.

7. Shwartzman, G.: Enhancing Effect of Cortisone upon Poliomyelitis Infection (Strain $MEF_1$) in Hamsters and Mice, Proc. Soc. Exper. Biol. & Med. **75**:835, 1950; Poliomyelitis Infection in Cortisone-Treated Hamsters Induced by the Intraperitoneal Route, ibid. **79**:573, 1952.

8. Aronson, S. M., and Shwartzman, G. Skeletal Muscle Lesions in Cortisone Enhanced Poliomyelitis, J. Neuropath. & Exper. Neurol. **12**:94, 1953.

## EXPERIMENTAL

The poliomyelitis virus used was the $MEF_1$ strain, kindly supplied by Dr. Peter K. Olitsky, and was maintained in this laboratory by serial intracerebral mouse passages. Virus pools were preserved in an electric refrigerator at —25 C. Male Syrian hamsters weighing 20 to 28 gm. were employed in all experiments. The animals were subdivided into experimental and control groups, as described in Table 1.

Clinical paralysis, verified histologically, was substantially greater in groups receiving cortisone (Table 1). The resultant infection was invariably more fulminant, and death was an unfailing consequence. A group of tests, outlined previously,[9] were periodically used to preclude the presence of any adventitious virus in the inoculum pools or the tissues studied.

Animals from all groups were killed at daily intervals. Tissues were fixed in 10% buffered formalin, Bouin's solution, and ice-cold acetone. A wide range of staining procedures was used.

TABLE 1.—*Effect of Cortisone on Poliomyelitis Infection in the Syrian Hamster Induced by Various Routes*

| Group | No. of Hamsters | Inoculum | Route* | Cortisone* | Paralysis, % | Mortality, % | Myositis |
|---|---|---|---|---|---|---|---|
| A | 40 | 0.05 cc. MEF1 stock mouse brain passage diluted 1:20† | I. C. | 5 mg., I. M., once with virus inoculation | 100 | 100 | Paravertebral muscles |
| B | 40 | 0.05 cc. MEF1 stock mouse brain passage diluted 1:20† | I. C. | None | 45 | 24.4 | ..... |
| C | 40 | 0.5 cc. MEF1 stock mouse brain passage diluted 1:20 | I. P. | 3 mg., I. M., 4 times; day prior to and day of virus inoculation | 100 | 100 | Paravertebral muscles |
| D | 40 | 0.5 cc. MEF1 stock mouse brain passage diluted 1:20 | I. P. | None | 0 | 0 | ..... |
| E | 60 | 0.2 cc. MEF1 stock mouse brain passage diluted 1:20 | I. M.‡ | 3 mg., I. M., 4 times; day prior to and day of virus inoculation | 100 | 100 | Site of virus inoculation |
| F | 20 | 0.2 cc. MEF1 stock mouse brain passage diluted 1:20 | I. M.‡ | None | 0 | 0 | ..... |

* I. C., intracerebral; I. P., intraperitoneal; I. M., intramuscular.
† Mouse medial lethal dose $10^{-8.7}$.
‡ Intramuscular injections of virus were made into front limbs and those of cortisone into hind limbs.

Within the time limits of the experiments (seven days), muscle lesions were observed only in animals receiving virus and cortisone (Groups A, C, E; Table 1). While microscopic myositis was a constant feature there was no ubiquity of lesions. The degenerative changes were confined to those striated muscle groups in closest proximity to the site of virus inoculation. The myositis following intracerebral inoculation was limited to the paravertebral musculature. Muscle changes after intramuscular inoculation were first observed in the immediate vicinity of the inoculation site. Following infection by the intraperitoneal route, the myositis was restricted to retroperitoneal muscles adjacent to the vertebral column. The time of emergence of myositis differed according to the route of viral administration. However, the microscopic picture was similar.

*Microscopic Observations.*—The earliest visible changes in affected muscles consisted of fiber separation and a tendency toward transverse fracturing (Fig. 1*A*). Zones within the sarcoplasm assumed a glistening hyaline-like appearance, some-

9. Shwartzman, G., and Fisher, A.: Alteration of Experimental Poliomyelitis Infection in the Syrian Hamster with the Aid of Cortisone, J. Exper. Med. **95**:347, 1952.

times characterized by a barely perceptible basophilic discoloration. The cross striations became obscured, and concurrently the longitudinal myofibrils appeared more prominent, coarser, and undulant. Scattered segments within each myofiber seemed to be affected more profoundly, showing coalescent areas of hyaline degen-

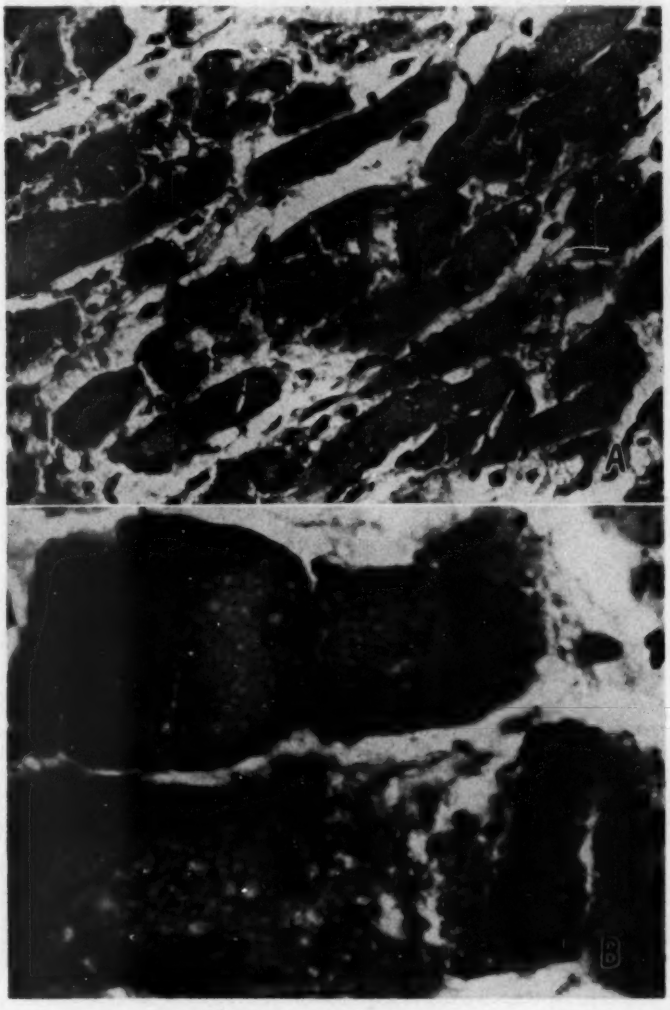


Fig. 1.—*A*, early stage of poliomyelitis myositis in the cortisone-prepared hamster showing fiber separation, edema, and some transverse fracturing. Hematoxylin and eosin; × 118. *B*, slightly later stage demonstrating zones of hyaline degeneration, lateral swelling of the affected fiber, and commencing transverse fracturing. Hematoxylin and eosin; × 1,040.

eration and lateral swelling (Fig. 1*B*). Such tuberous enlargements were widespread within 24 hours after the commencement of the degenerative process. At times the segmental swelling resulted in a fourfold increase in transverse diameter

of the muscle cell. Myofibrils tended to persist for a longer time than the cross-striations and a homogeneous substance appeared between these longitudinal structures (Fig. 2*A*). Disruption of the involved muscle fiber was initiated usually at

Fig. 2.—*A*, necrotic fiber showing linear configuration of intracellular basophilic substance. Note lack of any inflammatory response at this stage. Hematoxylin and eosin; × 1,040. *B*, reticulum stain showing relative preservation of sarcolemmal sheaths in the face of underlying sarcoplasmic necrosis. Degenerated muscle segments have retracted back, leaving empty intrasarcolemmal cavities. Laidlow stain; × 118.

the junctions where the globoid swellings commenced. Numerous globular masses were thus isolated from the parent fiber. Some degree of continuity was maintained, however, since the encompassing sarcolemmal sheath did not rupture until

later in the process (Fig. 2*B*). No internal architecture was evident within the sarcoplasmic globoids, except in the vicinity of the transverse fiber fracture lines, where a palisading or radiating linear configuration was seen. These internal struc-

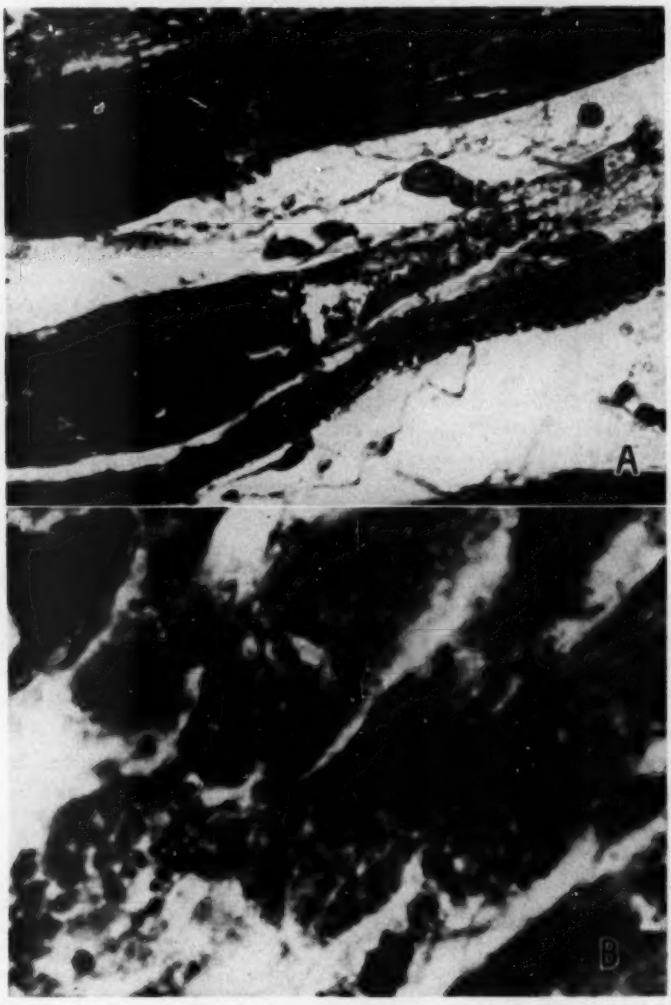


Fig. 3.—*A*, necrotic segment of involved fiber, with released basophilic granules confined to empty sarcolemmal space. Normal, apparently uninvolved, fibers in vicinity. Mallory phosphotungstic acid hematoxylin stain; × 504. *B*, basophilic granules with surrounding halos in necrotic segment. A few such granules have been discharged into the interstitium. Hematoxylin and eosin; × 1,040.

tures were evidently remnants of the basophilic substance previously deposited between the myofibrils. Under higher power it could be demonstrated that the basophilic bands were composed of discrete granules in linear arrangement. Variable

numbers of these basophilic spherules were released from the fractured terminals of larger globoid swellings and were seen filling the empty intrasarcolemmal cavities (Fig. 3). Muscle nuclei showed a slightly retarded degeneration, exhibiting pyknosis coincidentally with late hyaline alteration of the cytoplasm and karyolysis following basophilic necrosis. When fully developed, the stage of hyaline degradation was characterized by numerous rounded anuclear masses of friable, opaque, and glistening substance. The normal muscle birefringency displayed under polarized light was lacking at this stage. A striking feature was marked hematoxylinophilia exhibited by these anuclear masses, particularly with iron hematoxylin stains (Fig. 4*A*). The segments also showed an affinity for basic aniline dyes, such as methylene blue. The affected segments rapidly underwent granular necrosis with superimposed mineral deposition.

There was a greater tendency toward mineralization at fractured muscle ends and within the minute spherules lying free within the sarcolemmal confines. At this phase the sarcolemmal membrane often appeared severed and retracted back upon the crevised and fragmented ends of the involved segments.

A dilatory inflammatory response supervened. Leucocytes and mononuclear cells migrated through rents in the sarcolemmal sheath. Concurrently, the sarcolemmal cells became hyperplastic, rarely sufficiently, however, to obscure the fundamental underlying necrosis. Dissolution proceeded to a relatively late stage before mesodermal response became conspicuous. Neighboring blood vessels were similarly quiescent until late, again revealing the suppressive action of cortisone upon inflammation.[10] The entire process described above transpired within 48 hours. Regenerative changes were not seen, since animals died within a few days following onset of the disease.

Frozen sections of different phases of the myositis were stained with Sudan IV. Normal hamster muscle did not show any intracellular neutral fat by this technique. Muscle derived from hamsters treated with cortisone alone showed sudanophilic material in very small amounts occupying the intermyofibrillar spaces. This change could be demonstrated about 48 hours following cortisone inoculation. In animals receiving virus parenterally, in addition to cortisone, coalescence and increase in the intracellular lipid coincided with the onset of early degeneration (Fig. 4*B*). The linear configuration of the lipid persisted until disappearance of the limiting myofibrils. The radiating or parallel pattern at the fractured ends of the globular muscle segments described above was also prominently sudanophilic, as well as the discharged granules juxtaposed to the severed muscle ends (Fig. 5*A*).

The intracellular basophilia, with routine staining procedures, presented the tinctorial qualities of mineral deposition. Von Kossa's technique stained these foci black, indicating the presumptive presence of calcium (Fig. 5*B*). The alizarin

10. (*a*) Ragan, C.; Howes, E. L.; Plotz, C. M.; Meyer, V.; Blunt, J. W. and Lattes, R.: The Effect of ACTH and Cortisone on Connective Tissue, Bull. New York Acad. Med. **26**:251, 1950. (*b*) Moon, V. H., and Tershakovec, G. A.: Influence of Cortisone upon Acute Inflammation, Proc. Soc. Exper. Biol. & Med. **79**:63, 1952. (*c*) Aronson, S. M., and Shwartzman, G.: Histopathogenesis of Cortisone-Altered Experimental Poliomyelitis: Observations on the Syrian Hamster Inoculated Intracerebrally with Strain $MEF_1$, Am. J. Path. **29**:381, 1953.

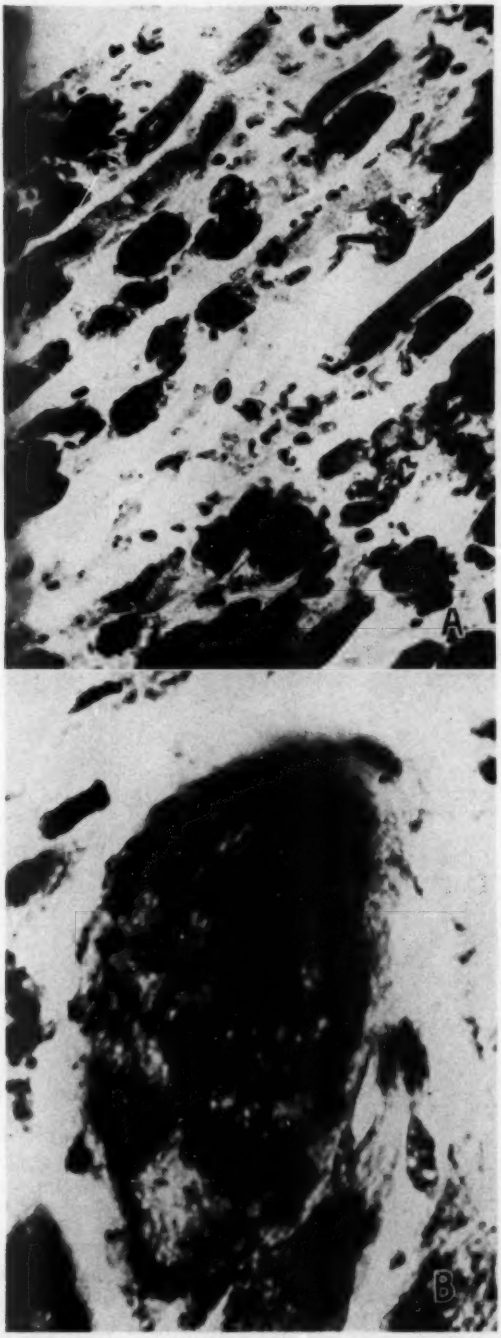


Fig. 4.—*A*, degenerating segments showing marked affinity for iron hematoxylin stain; × 87. *B*, frozen section of MEF, strain myositis stained with Sudan IV. Darker areas represent sudanophilic material. Note linear arrangement corresponding to basophilia seen in Figure 2*A*; × 1,040.

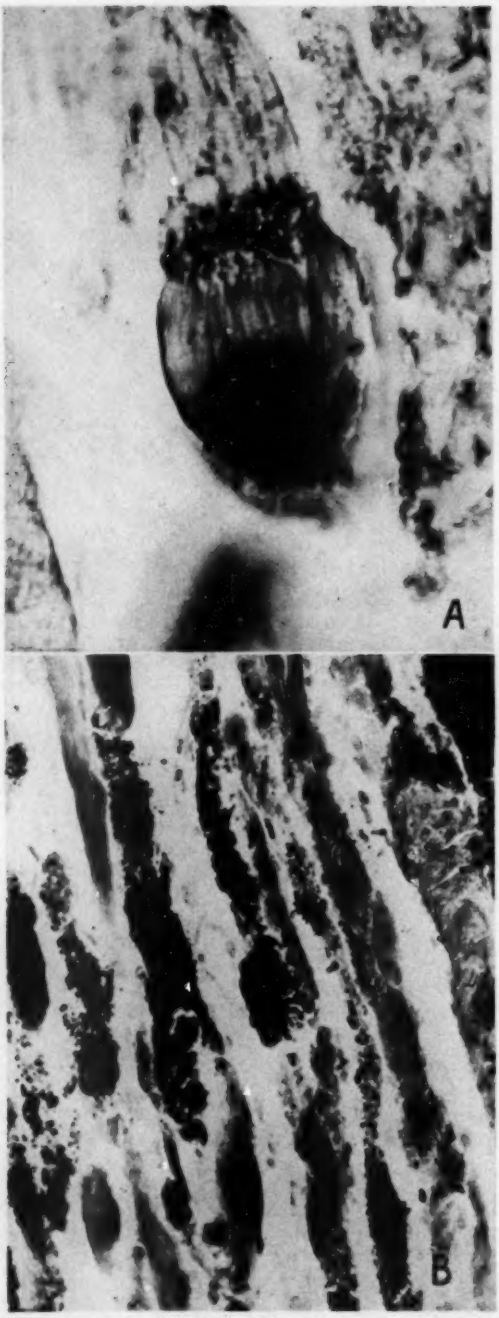


Fig. 5.—*A*, frozen section of involved muscle stained with Sudan IV. Note that there is a congregation of sudanophilic granules at transverse lines of fracture; × 504. *B*, von Kossa stain of $MEF_1$ strain myositis demonstrating granular foci for calcification; × 87.

red S test and a specific microchemical test, described by Bunting,[11] confirmed the presence of calcium. Gömöri's stain for iron was negative. It appeared that calcification occurred only in zones of lipid deposition and that necrosis of the fat was a necessary precursor of such mineralization.

The observed basophilic granules released from necrotic sarcoplasm merited further consideration, since similar microscopic particles have been described as a phase of Coxsackie virus myositis.[12] The particles seen in myositis caused by infection with $MEF_1$ stain of poliomyelitis virus varied in size from 0.5 to 1.0 $\mu$ and were invariably spherical in contour. When first observed, they were incorporated within the degenerative cytoplasm of the muscle cell and were usually evident in regions of more profound necrosis. At this stage the bodies imparted a minimal glassy appearance. Within a short time basophilia supervened, and with further myocellular degradation they were released into the surrounding interstitium. The particles were selectively stained by procedures for neutral lipid and calcium (Fig. 5); at times they were faintly fuchsinophilic. Positive reactions were obtained with the Giemsa and McManus periodic acid-leucofuchsin stains.

TABLE 2.—*Experimental Procedure for Control Studies*

| Group | No. of Hamsters | Inoculum, Cc.* | Route† | Cortisone† |
|---|---|---|---|---|
| G | 5 | 0.05 | I. C. | 5 mg., I. M., simultaneously with I. C. injection |
| H | 5 | 0.05 | I. C. | None |
| I | 5 | 0.5 | I. P. | 3 mg., I. M., 4 times; day prior to and day of I. P. inoculation |
| J | 5 | 0.5 | I. P. | None |
| K | 10 | 0.2 | I. M. | 3 mg., I. M., 4 times; day prior to and day of I. P. inoculation |
| L | 10 | 0.2 | I. M. | None |
| M | 110 | None | .... | 3 mg., I. M., 4 times, spaced over 2 days |

* Normal mouse brain emulsion diluted 1:20.
† I. C., intracerebral; I. P., intraperitoneal; I. M., intramuscular.

The Feulgen reaction for thymonucleic acids stained only nuclear residua in such degenerative cells but did not stain basophilic granules.

Histochemical studies delineating phosphatase activity by the Gömöri method [13] showed selective deposition of silver in specific foci of muscle necrosis. Normal muscle fibers were unstained by this procedure.

*Control Studies.*—Since two factors (i. e., virus and cortisone) were introduced into the experimental animal, the specificity of the resultant lesions remained to be proved. Cortisone acetate, in dosage comparable to that previously indicated, was administered to hamsters. These animals, in addition, received intracerebral, intraperitoneal, or intramuscular inoculations of normal mouse brain emulsion, as summarized in Table 2. Within the time period of the experiment, no degenerative features were demonstrable in muscles derived from numerous sites. A mild degree of atrophy was seen, exemplified by a slight diminution in cross sectional diameter; also, as mentioned above, an increase in stainable fat was noted. Animals retained for

11. Bunting, H.: Histochemical Analysis of Pathological Mineral Deposits at Various Sites; With Discussion of Methods Used, A. M. A. Arch. Path. **52**:458, 1951.

12. Pappenheimer, A. M.: Fuchsinophile Granules in the Tissues of Mice Infected with the Connecticut-5 Strain of Coxsackie Virus, J. Exper. Med. **95**:251, 1952.

13. Gömöri, G.: Microtechnical Demonstration of Phosphatase in Tissue Sections, Proc. Soc. Exper. Biol. & Med. **42**:23, 1939.

greater periods of time were also studied histologically. Three animals, killed 16 days after adminstration of cortisone, presented microscopic lesions similar in appearance to the above described myositis. Over 100 other cortisone-treated control hamsters failed to show such lesions. The foci of myositis in the three hamsters demonstrating this change bore no relationship to the site of mouse brain inoculation and were never as extensive as the myositis associated with parenteral inoculation of poliomyelitis virus.

Further studies were then undertaken in order to clarify the etiology of the hamster myositis and to determine its relation to the viral infection. A series of 57 male Syrian hamsters (20 to 28 gm.) were given an intramuscular injection of $MEF_1$ virus mouse brain emulsion in one upper limb and an injection of similar volume of normal mouse brain emulsion in the contralateral limb. All animals were given a course of cortisone. Animals were killed at daily intervals up to six days and studied histologically. The results are summarized in Table 3. No muscle lesions were observed in animals killed within 48 hours following virus inoculation. A typical myositis was found at the site of virus inoculation in cortisone-treated animals killed between 48 and 120 hours following intramuscular inoculation of $MEF_1$. After 120 hours a small percentage of animals showed, in addition, minimal scattered lesions in the limb where normal mouse brain emulsion was injected. These lesions were discrete and usually separated by broad areas

TABLE 3.—*Incidence, Location, and Severity of Histologic Lesions Following Intramuscular Inoculation of $MEF_1$ into Cortisone-Treated Hamsters*

| Hr. After Viral Inoculation | Limb Inoculated with Virus | | | | | | Limb Inoculated with Control Normal Mouse Brain Emulsion | | | | | % Showing Histological Evidence of Myelitis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of Hamsters | % Showing Myositis | Degree of Myositis* | | | | % Showing Myositis | Degree of Myositis | | | | |
| | | | 0 | + | ++ | +++ | | 0 | + | ++ | +++ | |
| 18 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 48 | 3 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| 72 | 15 | 86.6 | 2 | 8 | 5 | 0 | 0 | 15 | 0 | 0 | 0 | 30 |
| 96 | 24 | 95.8 | 1 | 5 | 13 | 5 | 0 | 24 | 0 | 0 | 0 | 76.9 |
| 120 | 5 | 100 | 0 | 1 | 3 | 1 | 20 | 4 | 1 | 0 | 0 | 100 |
| 144 | 7 | 85.7 | 1 | 2 | 3 | 1 | 28.5 | 5 | 2 | 0 | 0 | 100 |

* Degree of myositis determined by the following criteria: 0, no changes other than those incident to needle tract damage; +, early hyaline degeneration, loss of striation, fiber swellings, fiber separation; ++, transverse fracturing, basophilia, early necrosis; +++, diffuse involvement, extensive necrosis, and early inflammatory response.

of completely normal musculature. It was shown elsewhere [14] that dissemination of the virus, following intramuscular inoculation into a cortisone-prepared hamster, occurs beyond 96 hours and hence coincides with the emergence of minimal lesions in distant musculature seen in this series of hamsters beyond the fifth day.

In addition, attempts were made to induce myositis in mice. Adult mice, receiving 2 mg. of cortisone acetate a day for two days, were inoculated intramuscularly with 0.2 cc. of a 1:200 dilution of $MEF_1$ virus. Another group of adult mice similarly treated with cortisone were inoculated intramuscularly with the suckling mouse-adapted strain of $MEF_1$ diluted 1:100 (kindly supplied by Dr. J. Casals). Suckling mice were given injections subcutaneously with 0.25 mg. of cortisone acetate and inoculated intramuscularly with 0.05 cc. of a 1:200 dilution of the suckling-adapted strain of $MEF_1$. Animals from all groups were periodically killed and their tissues were studied microscopically. No myositis was demonstrated. Attempts to elicit a myositis with the neurotropic virus of herpes simplex (kindly supplied by Dr. B. Aronson) in cortisone-treated mice and hamsters were similarly unsuccessful.

With a wide spectrum of viral agents capable of eliciting an essentially similar muscle lesion in experimental animals,[6] the etiologic specificity of the alteration seemed in doubt. It was con-

14. Shwartzman, G., and Aronson, S. M.: Progression of Poliomyelitis Virus (Type 2) in Cortisone-Treated Hamsters Following Infection by Parenteral Routes, Fed. Proc. **12**:458, 1953.

ceivable that the sequence of changes described above represented the limited reactive capacity of striated muscle. It was believed to be of value, therefore, to study the microscopic picture in muscle resulting from the action of a deleterious agent other than viruses.

A strain of Paracolobactrum coliforme endemic to the enteric tract of hamsters was inoculated into the limb muscles of hamsters. Animals were killed at daily intervals and the involved musculature examined by routine histological methods. The response to the inoculated bacterial culture was prompt and exhuberant. The salient feature in all stages was interstitial exudation and organization. Muscle fibers separated and isolated by the encroaching suppuration were atrophied but usually failed to show any concomitant disintegration (Fig. 6*A*). The complex of segmental hyaline degeneration seen in viral lesions of muscle was not present. Fibroblastic proliferation and encapsulation of the interstitial abscess were prominent by the third day. In some central abscess areas muscle fibers were totally destroyed. In such areas evidence of regeneration was prompt. Fusiform myoblastic cells with numerous linearly oriented nuclei extended into the abscess. Within some of these nuclei, rounded homogeneous eosinophilic bodies surrounded by clear halos were occasionally seen (Fig. 6*B*). These inclusion bodies closely resembled structures seen in rodent myositis (Fig. 6*C*) caused by the GD VII encephalomyelitis virus.[2]

When the same bacterial inoculum was injected into cortisone-prepared hamsters, the exudation and encapsulation were markedly limited. Characteristically, the central portions of the lesion showed massive colonies of bacteria surrounded by acellular meshworks of fibrin. Muscle cells underwent early ischemic necrosis, but no segmentation or basophilia was apparent. Leucocyte infiltration was demonstrated only at the periphery of the altered area. Blood vessels were often the site of mural necrosis and fibrin thrombosis. The infection was widely disseminated, and death ensued within 96 hours.

## COMMENT

A series of changes essentially of an intracellular nature typifies the myositis attributable to the direct action of the poliomyelitis virus in hamsters treated with cortisone. The preliminary alterations are restricted to discrete segments of the muscle cell, often leaving the remaining sarcoplasm seemingly unaffected. A hyaline-like degeneration occurs in such segments, resulting in localized transverse distension. The more profoundly affected portions of the muscle fiber become detached and are seen as isolated spheres of necrotic sarcoplasm. Collaterally, large intracellular sudanophilic droplets appear, become variably calcified, and are released into the interstitium following fragmentation of the cell structure. The incremented fat content of normal hamster muscle, following administration of cortisone alone, suggests that this deposition may be a significant antecedent in the hamster to local viral propagation. Once muscle degeneration has commenced, the amount of sudanophilic material increases significantly. Intracellular lipid is an integral feature of numerous degenerative and dystrophic processes involving skeletal musculature [15] and is demonstrable experimentally following simple neurotomy.[16] Rather than

15. Adams, R. D.; Denny-Brown, D., and Pearson, C. M.: Diseases of Muscle, New York, Paul B. Hoeber, Inc., 1953.

16. Humoller, F. L.; Hatch, D., and McIntyre, A. R.: Effect of Neurotomy on Lipid Content of Striated Muscle, Am. J. Physiol. **169**:654, 1952.

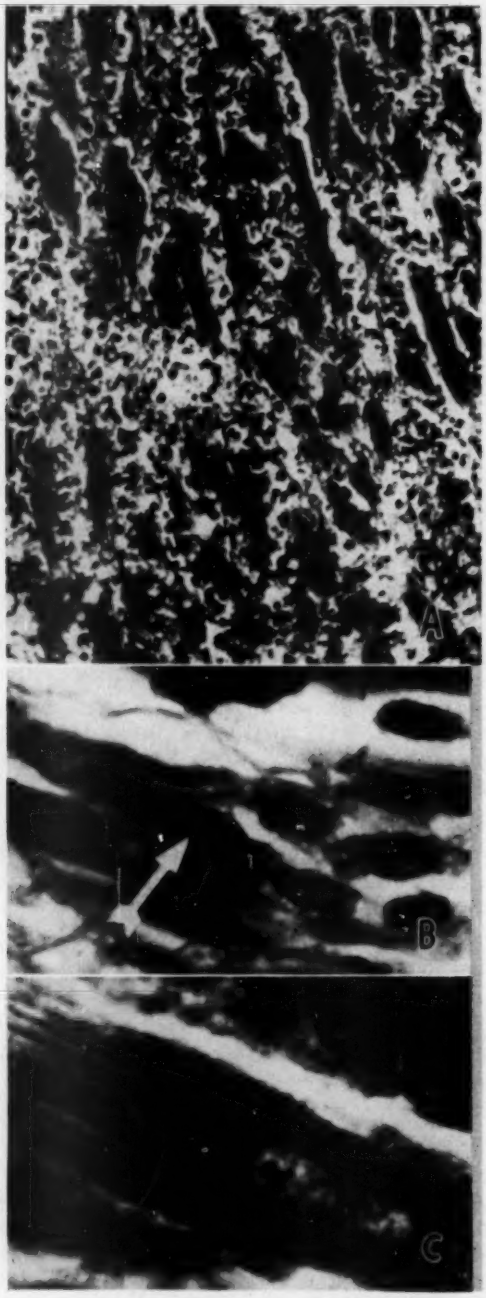


Fig. 6.—*A*, myositis caused by intramuscular inoculation of Paracolobactrum coliforme. Extensive interstitial exudation causing fiber separation and some atrophy but no segmental necrosis. Hematoxylin and eosin; × 87. *B*, regenerating muscle fibers in region of bacterial abscess. Arrow points to intranuclear inclusion body. Hematoxylin and eosin; ×1,300. *C*, inclusion body associated with myositis caused by intramuscular inoculation of GD VII virus. Hematoxylin and eosin; × 1,300. (Slide obtained through the courtesy of Dr. A. M. Pappenheimer.)

representing a precedent substrate, it would appear that the emergence of intracellular lipid in large amounts denotes an end-result of the noxious capacities of the virus. This concept is strengthened by the demonstration of poliomyelitis virus proliferation in hamster muscle before any notable morphological changes take place.[17]

Calcification, which is so prominently displayed in the fully expressed muscle lesion, is intimately linked with preceding fat deposition. Necrosis of this lipid apparently forms the nidus for the dystrophic mineralization. As in many instances of calcium salt deposition in devitalized tissue, the process is not a function of heightened blood calcium levels but is secondary to a local biochemical transformation favoring calcium precipitation.[18]

The muscle lesions in the hamster are basically equivalent to those caused by other murine neurotropic viruses (Fig. 7). There is a particularly striking similarity to Coxsackie infection of the suckling mouse. Identical segmental hyaline degeneration and necrosis are demonstrable. A few differences can be distinguished. Mineralization of affected segments is seen in both instances, but, while iron and calcium constitute the mineral deposits in Coxsackie disease of muscle, only calcium can be found in the myositis of poliomyelitis etiology. Regenerative attempts are prominently featured in Coxsackie [1a,b] and GD VII virus [2] myositis. In the case of the former, reparation is first noted as early as one day following commencement of overt disease and in GD VII muscle lesions by the fourth day. Little or no regeneration is demonstrable in poliomyelitis myopathy. This may be explained in part by the more fulminant nature of cortisone-enhanced experimental poliomyelitis. Death may occur as early as the third day after viral inoculation. The role of cortisone in inhibiting regrowth of the remaining cellular elements in the involved area must also be appreciated. Fibroblastic responsiveness has been shown to be depressed by cortisone.[10a] Myoblastic reaction may possibly suffer a similar fate.

Pappenheimer has described small fuchsinophilic bodies (F granules) in the vicinity of muscle cells infected with Coxsackie virus.[12] It was his impression that these cytoplasmic granules were intimately related to virus activity, perhaps representing structures akin to elementary bodies. Similar bodies, described above, are associated with the myositis of experimental poliomyelitis. From the information derived from our studies, it is believed that such bodies represent the ultimately extruded products of cytoplasmic disintegration, commencing as intermyofibrillar lipid globules. Increased alkaline phosphatase activity is centered about these granules, but this finding is difficult to evaluate. The fuchsinophilic staining reaction of the fuchsinophilic bodies in Coxsackie myositis was only poorly displayed in the poliomyelitis myositis, and hence it is possible that the granules of these diseases may represent different entities.

No inclusion bodies were seen in the myositis of poliomyelitis. However, intranuclear eosinophilic structures resembling those noted in GD VII virus myositis were seen in the regenerating muscle cells of bacterial myositis. It can be conjectured that the bacterial infection activated a latent viral infection in the hamster, thus explaining the inclusion bodies, but the absence of primary muscle necrosis and the coincidence of these bodies restricted to sites of regeneration would indicate

17. Shwartzman, G., and Aronson, S. M.: Poliomyelitis Infection by Parenteral Routes Made Possible by Cortisone, Ann. New York Acad. Sc. **56**:793, 1953.

18. Anderson, W.: Pathology, St. Louis, C. V. Mosby Company, 1948.

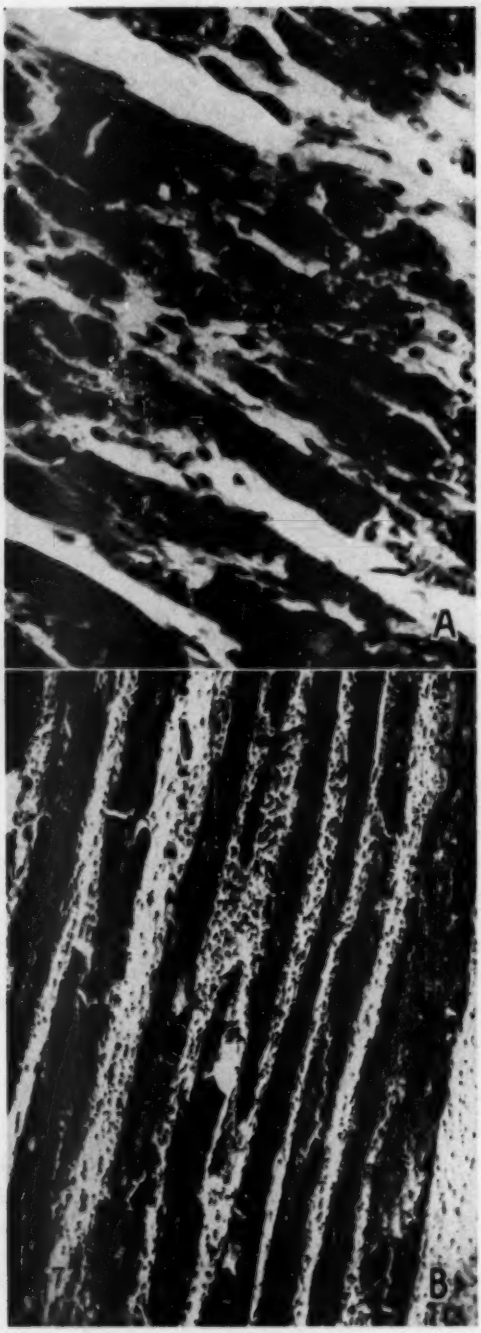


Fig. 7.—*A*, Coxsackie myositis in the suckling mouse. Note resemblance to features of poliomyelitis myositis. Hematoxylin and eosin; × 118. (Tissues obtained through the courtesy of Dr. J. Melnick.) *B*, myositis produced in suckling mice following intracerebral inoculation of DA virus. Inflammatory response more prominent than displayed in Figures 1 to 3. Hematoxylin and eosin; × 87. (Slide obtained through the kindness of Dr. A. M. Pappenheimer.)

the inadequacy of this surmise. Inclusion bodies are not invariable evidence of viral infection [19] and, as in the present instance, appear to denote a morphological phase of cellular reconstitution.

Because of the nonspecific disease-enhancing properties of cortisone [20] a large number of control hamsters, receiving only cortisone, were also studied. Three such animals presented minimal microscopic lesions of muscle similar to those regularly observed following parenteral inoculation of the $MEF_1$ virus into cortisone-treated hamsters. In order to determine whether the myositis in these three hamsters represented a metabolic result of cortisone inoculation or the activation of a latent infection in some hamsters, the following experiment was carried out: Cortisone-treated hamsters were inoculated with the $MEF_1$ virus in one limb and with normal mouse brain emulsion in the contralateral limb. Myositis promptly developed at the site of viral inoculation. It is apparent that numerous agents have the capability of initiating a myositis in the hamster, one of these being the $MEF_1$ strain of poliomyelitis. In a further publication, correlation between foci of myositis and proliferation of the poliomyelitis virus will be described.

Myositis of bacterial origin is fundamentally an interstitial phenomenon. The muscle cells show secondary atrophy and occasionally total necrosis. Preliminary cortisone administration enhances the infection, restrains mesodermal response, and permits bacterial dissemination but does not elicit changes similar to those seen with viral infections. The salient property of primary intracellular segmental necrosis is singularly observed in muscle lesions created by viruses and hence can be considered as the most reliable microscopic criterion of viral myositis.

The virus of poliomyelitis has been separated in the past from other neurotropic viruses as endowed with a highly selective neurotropism. Within recent years the lack of viscerotropism was considered characteristic of the poliomyelitis virus.[21] The findings recorded above would indicate a broader tissue affinity for the virus of poliomyelitis and hence a closer relationship to other viruses exhibiting the binary potentiality of myoneurotropism in the appropriate host.

SUMMARY

The $MEF_1$ strain of poliomyelitis virus, inoculated into cortisone-prepared Syrian hamsters, is capable of initiating a florid necrotizing myositis. The microscopic lesions are characterized by rapidly progressing hyaline degeneration, loss of striations, edema, extensive necrosis, and mineralization. The resultant microscopic picture is basically similar to that evoked by numerous other neurotropic viruses in experimental animals. Evidence is presented elsewhere that there is a proliferation of the poliomyelitis virus in such sites.

The histological sections were prepared by Mrs. M. Pirogow, and Mr. H. Fischler provided the photomicrographs.

19. Olitsky, P. K., and Hartford, C. G.: Intranuclear Inclusion Bodies in the Tissue Reactions Produced by Injections of Certain Foreign Substances, Am. J. Path. **13**:729, 1937.

20. Antopol, W., and Quittner, H.: The Changing Pattern of Infectious Processes Under the Influence of Cortisone, J. Mt. Sinai Hosp. **19**:91, 1952.

21. Committee on Nomenclature: A Proposed Provisional Definition of Poliomyelitis Virus, Science **109**:701, 1949.

# ACTION OF PHOSELITE ON ALLOXAN DIABETES IN RATS AND RABBITS

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REPORTS indicate that Phoselite (sodium α-hydroxybenzylphosphinate) is efficacious in certain disorders in man due to its ability to stimulate the synthesis of glycogen and to favor the assimilation of carbohydrates. Some of the conditions in which its use has been recorded as having a salutary effect are (*a*) postirradiation sickness,[1] (*b*) convalescent period in surgical conditions and bone fracture,[2] (*c*) epidemic hepatitis,[3] and (*d*) prevention of cyanosis caused by sulfonamides (sulfapyridine).[4] It has also been found by Swiss workers that the drug at least partially prevented and/or cured the diabetic state caused by the injection of alloxan monohydrate in rabbits and rats.[5] Since, in view of the latter, such a compound might be invaluable in its application to human diabetes, the following experiments were designed to confirm the results of the Swiss workers in connection with alloxan diabetes. Generally they were patterned on the Swiss methods but specifically were more detailed.

## METHODS AND RESULTS

Ninety-eight male albino rats and 92 albino rabbits, of which only 4 were females, were used. At the time of acquisition the rabbits weighed approximately 2,000 gm., the rats 150 gm. All were kept in the departmental experimental room, were fed appropriate Purina Chow Checkers,[6] and were allowed unlimited amounts of tap water.

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This work was supported by a research grant from V. Siegfried Limited, Zofingen, Switzerland, and from Thomas Leeming and Company, Inc., New York.

1. Hohl, K.: Use of the Sodium Salt of α-Oxybenzylphosphinic Acid in Long Lasting X-Ray Treatments, Praxis **38**:568, 1949.

2. Goecke, C. A.: Experiences in the Treatment of Surgical Diseases with a Recently Evaluated Group of Organically Bound Phosphorus Substances, Deutsche med. Rundschau **18**:512, 1949.

3. Moser, P.: Experimental Findings in the Action of Sodium α-Oxybenzylphosphinate, München. med. Wchnschr. **93**:372, 1951.

4. Moser, P.: Treatment of Capillary Cyanosis Following Sulfonamide Therapy, Med. Welt **20**:91, 1951.

5. V. Siegfried Limited, Zofingen, Switzerland, personal communication to the authors.

6. Purina Dog Chow Checkers were obtained from the Ralston Purina Company, St. Louis, Mo., and were composed of crude protein 24% (minimum), crude fat 5% (minimum), crude fiber 5% (maximum), nitrogen-free extract 46% (minimum). The ingredients include meat meal, dried skimmed milk, wheat germ, dried beef pulp, cooked corn grits, cereal feed (from

*(Footnote continued on next page)*

Generally the experiment consisted of the injection of alloxan monohydrate,[7] followed later by administration of Phoselite.[8] Although we were cognizant of the fact that in a higher percentage of animals permanent diabetes develops when alloxan monohydrate is given in doses of 200 mg. per kilogram of body weight [9] after a period of fasting, we gave nonfasting animals somewhat smaller doses throughout the experiments because we desired a large number of surviving diabetic animals for study We believed that, although there might be a higher percentage of animals in which diabetes would develop, there would be a corresponding higher rate of mortality from hypoglycemia if fasting animals and the higher doses were used.

All animals in the Phoselite-treated groups received daily subcutaneous injections of 5% aqueous solution of Phoselite until death (600 mg. per kilogram of body weight for the rabbits and 300 mg. per kilogram of body weight for the rats). Approximately three weeks after the injection of alloxan monohydrate, surviving animals treated with Phoselite and nondiabetic control animals were killed. Sections taken from the pancreas, heart, lungs, liver, kidneys, testes or ovaries, bone marrow, brain, and skin (at the site of Phoselite injection) were fixed in Bouin's solution and stained routinely with hematoxylin and eosin. Sections of pancreas were also stained by the Gömöri technique.[10]

Blood sugar levels (micro-Benedict method [11]) in the animals, following injection of alloxan monohydrate, fell into three distinct categories, permitting the classification of the animals as permanently diabetic, transitorily diabetic, and nondiabetic. In the two species the permanently diabetic animal showed elevation of the blood sugar to levels of 180 mg. per 100 cc. or higher during the course of observation after the initial hyperhypoglycemic phase. In addition, urine studies in the rabbits disclosed without exception positive findings for sugar and acetone (clinitest and acetest methods [12]) as determined at regular intervals throughout the course of observation. In those classified as transitorily diabetic, the blood sugar levels exceeded 180 mg. per 100 cc. for varying periods, followed by a gradual reduction to within the normal range of 60 to 120 mg. per 100 cc. Urinalysis in these rabbits disclosed glycosuria and acetonuria only during the period of hyperglycemia. The nondiabetic animals were those failing to show elevation of the blood sugar level to 180 mg. per 100 cc. at any time after the administration of alloxan monohydrate. The urines were at all times free of glucose and acetone.

Although the level of 180 mg. per 100 cc. was chosen as the lower limit by which the animals were categorized, actually all of the nondiabetic animals had an average blood sugar level of 100 mg. per 100 cc. In rats, as demonstrated by Lazarow and Palay,[13] blood sugar normally ranges from 90 to 160 mg. per 100 cc. In our animals preliminary blood sugar studies revealed normal levels ranging from 60 to 120 mg. in the rats and from 60 to 140 mg. in the rabbits. It was believed that by using 180 mg. as the maximal level of normal we would have a valid division of the animals into diabetic and nondiabetic.

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corn, wheat, and rice), soybean oil meal, dehydrated cheese, molasses, vitamin $B_{12}$ feed supplement, condensed fish solubles, riboflavin supplement, brewer's dried yeast, vitamin A and D feeding oils, thiamine, niacin, 2% steamed bone meal, 15% iodized salt. Purina Rabbit Chow Checkers (Complete Ration) E were obtained from the Ralston Purina Company, St. Louis, Mo., and were composed of crude protein 15% (minimum), crude fat 2% (minimum), crude fiber 18% (maximum), N. F. E. 46% (minimum). The ingredients include ground oats, ground yellow corn, dehydrated alfalfa meal, soybean oil meal, linseed oil meal, corn germ meal, alfalfa meal, wheat gray midlings, molasses, riboflavin supplement, vitamin D-activated plant sterol, 1.5% calcium carbonate, 1.5% low fluorine rock phosphate, 0.25% iodized salt.

7. This was obtained from Distillation Products Industries, Lot No. 1722.

8. This was obtained from V. Siegfried Limited, Zofingen, Switzerland.

9. Kass, E. H., and Waisbren, B. A.: A Method for Consistent Induction of Chronic Hyperglycemia with Alloxan, Proc. Soc. Exper. Biol. & Med. **60**:303, 1945.

10. Gömöri, G.: Observations with Differential Stains on Human Islet of Langerhans, Am. J. Path. **17**:395, 1941.

11. Hawk, P. B.; Bergeim, O.; Oser, B. L., and Cole, A. G.: Practical Physiological Chemistry, Ed. 11, Philadelphia, The Blakiston Company, 1937, p. 436.

12. Clinitest and Acetest materials were obtained from Ames Company, Inc., Elkhart, Ind.

13. Lazarow, A., and Palay, S. L.: The Production and Course of Alloxan Diabetes in Rat, J. Lab. & Clin. Med. **31**:1004, 1946.

The study was divided into its component parts because we were interested in determining the various means by which Phoselite possibly could alleviate alloxan diabetes as claimed by the Swiss investigators.

EXPERIMENT 1.—Alloxan monohydrate and Phoselite were given simultaneously at different sites. This was done to determine whether or not there might be an immediate protective action of Phoselite on the pancreas against alloxan. As indicated below, there was no appreciable difference between the animals receiving alloxan monohydrate and Phoselite and those receiving alloxan monohydrate alone.

*Rabbits.*—Fifteen animals were given alloxan monohydrate intravenously in doses of 175 mg. per kilogram of body weight (5% aqueous solution buffered to a pH of 5.5), followed immediately by an intravenous injection of an aqueous 10% solution of Phoselite in doses of 600 mg. per kilogram of body weight. Six of the animals died in hypoglycemia within 24 hours after the injection of alloxan monohydrate. One was found dead five days after the injection of alloxan monohydrate, and eight were ultimately killed three weeks after the injection of alloxan monohydrate. Of the nine surviving the initial 24-hour period, eight had permanent diabetes (blood sugars of 200 to 554 mg. per 100 cc.). This included the animal dying on the fifth day. One surviving animal did not have diabetes.

Fourteen animals serving as controls received only the designated amount of alloxan monohydrates. Three animals died within 24 hours of hypoglycemia; two died four and six days after the alloxan monohydrate injection; three were killed at the end of the three-week period, and six were entered as being under Experiment 4, which is mentioned below. Of the 11 that survived the initial 24 hours, 8 had permanent diabetes (blood sugars of 200 to 650 mg. 100 cc.). One had transitory diabetes (maximal blood sugar of 300 mg. per 100 cc.). Two did not have diabetes.

*Rats.*—Sixteen animals were given the alloxan monohydrate subcutaneously in doses of 175 mg. per kilogram of body weight (5% aqueous solution buffered to a pH of 5.5). This was followed immediately by subcutaneous injection of 5% aqueous solution of Phoselite in doses of 300 mg. per kilogram of body weight. None of the 16 animals died from hypoglycemia. Two had permanent diabetes (blood sugars of 180 to 378 mg. per 100 cc.). Fourteen had transitory diabetes (maximal blood sugars of 400 mg. per 100 cc.). There were no nondiabetic animals in this series.

Sixteen control animals were given equivalent doses of alloxan monohydrate only. There were no deaths from hypoglycemia. Three animals had permanent diabetes (blood sugars of 180 to 402 mg. per 100 cc.). Thirteen had transitory diabetes (maximal blood sugars of 425 mg. per 100 cc.).

EXPERIMENT 2.—Alloxan monohydrate and Phoselite were given together in an aqueous mixture to the treated group of animals. This was done to determine whether Phoselite had an effect on the diabetogenic property of alloxan, either chemically or physiologically. As indicated by the results of this experiment, there was no appreciable difference between the treated and the control rabbits. In the rats, however, there was a higher incidence of nondiabetic animals among the treated as compared with the control group. Thus, under conditions of this experiment Phoselite has some protective action in the rat. The precise mechanism involved remains unknown, but since the combination of Phoselite and alloxan monohydrate maintains the diabetogenic property of alloxan for the rabbits, a chemical reaction can be ruled out.

*Rabbits.*—Fifteen animals were given intravenously a mixture of aqueous 10% Phoselite and 5% alloxan monohydrate buffered to a pH of 5.5 so that each animal received 175 mg. of alloxan monohydrate per kilogram of body weight and 600 mg. of Phoselite per kilogram of body weight. Eight animals died within the 24-hour period of hypoglycemia; one died on the fifth day, and six lived to be killed three weeks after the injections of alloxan monohydrate. Of the seven that survived the initial period, all had permanent diabetes (blood sugars of 192 to 800 mg. per 100 cc.).

Fifteen animals serving as controls received only the designated amount of alloxan monohydrate. Seven animals died within the first 24 hours; two died four to six days after the injection of alloxan monohydrate; one nondiabetic animal was killed at the end of the experiment.

and five were again designated as animals in Experiment 4. Of the eight animals that survived the initial 24-hour period, seven were permanently diabetic (blood sugars of 220 to 650 mg. per 100 cc.). One animal did not have diabetes.

*Rats.*—Sixteen animals were given a subcutaneous injection of the mixture of aqueous 5% alloxan monohydrate and 5% Phoselite so as to receive 180 mg. of alloxan monohydrate per kilogram of body weight and 300 mg. of Phoselite per kilogram of body weight. Of the 16 animals there was one death on the sixth day after injection of alloxan monohydrate. One had transitory diabetes, and 15 did not become diabetic. Sixteen control animals again received only alloxan monohydrate. Five of these animals died, four on the second day and one on the third day after alloxan monohydrate administration. Of the remainder, seven were permanently diabetic, three transitorily diabetic, and one nondiabetic. The permanently diabetic animals showed blood sugar levels ranging from 190 to 676 mg. per 100 cc., the transitorily diabetic maximal levels of 380 mg. per 100 cc.

EXPERIMENT 3.—Phoselite was administered by mouth and subcutaneously to the treated group of animals for a period of 14 to 16 days prior to administration of alloxan monohydrate. This was carried out to insure maximal protective action of Phoselite (if present) in the animals prior to injection of alloxan monohydrate. As indicated by the results, there was no significant difference in the number of animals having diabetes in the treated and control groups.

*Rabbits.*—Fifteen animals received daily subcutaneous injections of aqueous 5% Phoselite in doses of 600 mg. per kilogram of body weight and a total of 30 to 65 gm. of powdered Phoselite in their drinking water. The Phoselite in the drinking water was given in a final dilution of 1%, higher concentrations having acted as a cathartic. The rabbits received the Phoselite for a period of 14 days, at which time they and 15 control animals were given intravenous injections of buffered aqueous 5% alloxan monohydrate in doses of 175 mg. per kilogram of body weight. All treated animals were given unlimited amounts of 1% aqueous solution of Phoselite as drinking fluid in addition to the usual daily subcutaneous injections until death. Of the treated rabbits, one died in three days, and four died five days after the alloxan monohydrate injection. Nine lived to be killed at the end of the experiment. Of the 14 that survived the initial 24-hour period, 10 were permanently diabetic (blood sugars of 205 to 650 mg. per 100 cc.). Three were transitorily diabetic (maximal blood sugars of 470 mg. per 100 cc.). One animal did not have diabetes. In the control group, five died within the first 24 hours after the injection of alloxan monohydrate; one died four days after the injection of alloxan monohydrate; three nondiabetic animals were killed at the end of the experiment, and five were made members of Experiment 4. Of the 10 that survived the initial 24-hour period, 6 had permanent diabetes (blood sugars of 205 to 650 mg. per 100 cc.). One animal had a transitory diabetes (maximal blood sugars of 295 mg. per 100 cc.). Three animals did not have diabetes.

*Rats.*—Eighteen animals were given Phoselite in their drinking water in 1% concentration and, in addition, subcutaneous injections of 5% aqueous solution of Phoselite in doses of 300 mg. per kilogram of body weight for 16 days. They and 16 control animals were given buffered aqueous 5% alloxan monohydrate in doses of 180 mg. per kilogram of body weight subcutaneously. All treated animals were given unlimited amounts of 1% aqueous solution of Phoselite as drinking fluid in addition to the usual daily subcutaneous injections until death. Of the 18 treated animals, 3 died—one on the third, another on the fourth, and the last on the fifth day after subcutaneous administration of alloxan monohydrate. Of the remaining 15 animals, 8 were permanently diabetic, 4 transitorily diabetic, and 3 nondiabetic. The diabetic animals showed blood sugar levels ranging from 190 to 330 mg. per 100 cc., the transitorily diabetics with maximal levels of 261 mg. per 100 cc. Of the 16 control animals, 2 died, one on the third and the other on the fourth day after alloxan monohydrate. Of the remainder, six were permanently diabetic; four were transitorily diabetic, and four were nondiabetic. The permanently diabetic animals showed blood sugar levels ranging from 180 to 543 mg. per 100 cc., the transitorily diabetic maximal levels of 291 mg. per 100 cc.

EXPERIMENT 4.—This experiment was limited to the rabbit and was designed to determine whether or not Phoselite could alleviate already established alloxan diabetes. The animals used in this group consisted of the 16 control animals which were rendered permanently diabetic in each of the first 3 experiments. Nine received 600 mg. per kilogram of body weight of 5% Phoselite subcutaneously for a period of approximately three weeks, while seven were kept

untreated in their diabetic state. Studies of the blood sugar level of those animals given Phoselite disclosed no reduction in the hyperglycemic level as compared with those in which Phoselite was withheld. All animals maintained the high blood sugar levels that developed after the injection of the alloxan monohydrate. Moreover, studies of the urine revealed persistence of glycosuria and acetonuria in all animals in the two groups.

In the experiments as a whole, weights of the individual rabbits and rats were recorded at regular intervals. In the rabbits there was no significant weight difference in the treated and control group of animals. Similar findings were generally noted in the rats, except those in Experiment 2. The large number of nondiabetic animals found in the treated group of this experiment showed an average weight gain of 60 gm. as compared to 33 gm. in the surviving control group.

Measurement of the water intake was carried out only in rabbits, as studies were not feasible in the rats under the conditions of the experiment. The water intake, while being rather constant in an individual rabbit, showed wide fluctuations when compared animal for animal. It was noted, however, that the diabetic animals showed a definite increased intake (600 cc. daily) when compared to the nondiabetic animals (200 cc. daily). Polyuria was present in the diabetic group as evidenced by large amounts of urine collected in the drip pans.

No detailed toxicologic studies were carried out to determine the toxicity of Phoselite. However, three rabbits not included in the groups mentioned above were given Phoselite subcutaneously and orally in amounts which totaled 112 gm. during the course of observation of 34 days. The average weight of each animal before the experiment was approximately 2.2 gm., and the average weight at the time of death was 2.8 kg. Also, in an effort to combat hypoglycemic convulsions, as many as 20 gm. of Phoselite per hour were administered subcutaneously to the Phoselite-treated rabbits in the first 3 experiments. Clinical observation, including periodic blood counts of all animals and histopathologic studies of the various organs of all of these rabbits, revealed no alterations to suggest toxicity. Histopathologic studies of many tissues, particularly the bone marrow, liver, kidneys, and brain of all other animals given Phoselite, also revealed no changes suggestive of toxicity of Phoselite. The only significant pathologic changes found in some of the animals were referable to the effects of alloxan monohydrate or complications of the ensuing diabetes. These include (*a*) disappearance of the beta cells of the islets of Langerhans,[14] (*b*) periportal hepatic necrosis,[15] (*c*) focal necrosis and calcification of the renal tubules,[15a] (*d*) pulmonary congestion and edema, and (*e*) bronchopneumonia.

## CONCLUSION

Under the conditions of our experiments, Phoselite (sodium α-hydroxybenzylphosphinate) had no effect in inhibiting or alleviating alloxan diabetes in the rabbit and rat when the two compounds were administered simultaneously but in separate areas of the body and when Phoselite preceded the administration of alloxan monohydrate. Mixing Phoselite with alloxan monohydrate before injection also showed no effect in inhibiting or alleviating diabetes in the rabbit but resulted in a decreased incidence of diabetes in the rat. Histologic studies of sections of the various organs showed only those changes attributable to alloxan diabetes. In spite of relatively high dosages, no clinical or histologic manifestations of toxicity from the Phoselite were observed.

Dr. Peter A. Herbut gave helpful suggestions in the preparation of this work.

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15. (*a*) Herbut, P. A.; Watson, J. S., and Perkins, E.: Hepatic and Renal Necrosis in Alloxan Diabetes of Rabbits, Arch. Path. **41**:516, 1946. (*b*) Lazarow and Palay.[18]

# PATHOLOGIC ANATOMY OF CORONARY HEART DISEASE

## Particular Reference to Cardiac Muscle Bundles

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THE DATA presented in this paper deal primarily with the normal and pathologic anatomy of the coronary circulation. They were obtained by a combined injection-dissection-cross section technique designed to (*a*) demonstrate the normal and abnormal patterns of coronary circulation, (*b*) determine accurately the blood supply of the muscle bundles, and (*c*) support these observations by a study of the distribution of myocardial damage due to coronary narrowing or occlusion.

This approach is new only insofar as it combines the advantages which an injection technique offers in studying the state of the coronary arteries with data referable to muscle bundles, which can only be obtained from the study of cross section slices. In addition, a simple technique is utilized for the injection studies, the simplicity of which it is hoped will encourage its adoption for the routine study of postmortem hearts.

Schlesinger [1] has made a definitive and detailed study of the coronary arteries, which remains as a classic in this field. However, he did not correlate his findings to the myocardial bundles, since he studied the relationship of coronary disease to myocardial damage in the "unrolled" heart. On the other hand, the previous data published on lesions in cardiac muscle bundles was obtained only by dissection. An earlier attempt to determine the blood supply of the bundles by dissection of these and the arteries supplying them convinced us that such data could only be obtained by a combination of dissection plus injection. Definitive information on the blood supply of the bundles is needed in order to clarify the nature of muscle bundle involvement emphasized by numerous authors.[2]

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From the Laboratories of the Marshfield Clinic and St. Joseph's Hospital and the Marshfield Clinic Research Foundation.

1. Schlesinger, M. J.: An Injection Plus Dissection Study of Coronary Occlusions and Anastomoses, Am. Heart J. **15**:528, 1938.

2. (*a*) Robb, J. S., and Robb, R. C.: Localization of Cardiac Infarcts in Man: I. A Comparison of Anterior-Posterior with Muscle Bundle Modes of Localization, Am. J. M. Sc. **197**:7, 1939; (*b*) Localization of Cardiac Infarcts in Man: II. Twenty-Nine New Cases of Muscle Bundle Localization with Postmortem Confirmation, ibid. **197**:18, 1939. (*c*) Lowe, T. E.: Significance of Myocardial Scars in the Human Heart, J. Path. & Bact. **49**:195, 1939; (*d*) Pathology of Coronary Ischemia, M. J. Australia **2**:491, 1939; (*e*) Note on the Musculature of Human Heart as Illustrated by Pathological Processes, ibid. **1**:826, 1940. (*f*) Lowe, T. E.,

*(Footnote continued on next page)*

Finally, simple dissection of the heart and coronary arteries as usually performed at autopsy leaves much to be desired from the technical standpoint, while yielding little information as to possible hemodynamic alterations in the coronary circulation as a whole. Although previously advocated injection techniques [1] are of themselves excellent, nevertheless they have not been widely adopted as routine procedure, possibly because of the lack of simplicity of apparatus and procedure. We do not claim that the method we have adopted is superior, but we have found it to be at least as good as the others and simple enough to use routinely in the autopsy room.

## MATERIALS AND METHODS

Hearts were obtained from 81 consecutive autopsies, from patients varying in age from newborn infants to 93 years. The heart was removed in the standard fashion, with particular care to sever only the tributary vessels and to avoid damage to the auricles. Weights recorded were for the fresh specimens.

After trimming away all but about 2 cm. of the aortic root, the coronary ostia could be easily inspected. Glass cannulae were carefully inserted into the ostia and tied firmly in place with a ligature placed as near as possible to the origin of the artery in order to avoid branches which are at times given off within a very short distance of the main orifice. Occasionally two adjacent ostia were found on one side, and the two were cannulated so that all branches were injected.

The injection medium was prepared by thoroughly mixing 400 gm. of x-ray barium (contains 90% $BaSO_4$ and 10% inert suspension agent) with 700 ml. of water. The suspension can be colored with dyes [1] so that each artery can receive material of contrasting colors. The injection apparatus consisted of two enema cans, one for each color, with a suitable length of rubber tubing and pinch clamp.

After the air was expressed from the rubber tubing and displaced from the glass cannulae, the rubber tubing was slipped over the cannula, the pinch clamp removed, and the can elevated to a height equivalent to a pressure of 150 mm. Hg. Injection was allowed to proceed for about five minutes, at which time the rubber tube was firmly tied just above the cannula and cut above the ligature to prevent backflow of barium from the vessels.

After the injection the uncut heart was x-rayed. When desirable routine dissection could be carried out, the roentgenogram was used as a guide. The technical factors used were the following: 100 ma., 1/10 second at 37 in. (93.98 cm.), 500 ma. rotating anode tube, 38 to 46 kvp, ordinary cassette. For our studies the heart was fixed for 7 to 10 days in 10% formalin and then sliced like a loaf of bread, beginning at the apex, the slices being about 1 to 1.5 cm. thick.[2h] In some instances the heart was cut along other planes in order to demonstrate better the distribution of a given vessel. The slices were then also x-rayed (100 ma., 1/20 second), and accurate outline drawings of the slices were made. Abnormalities noted in the vessels or the heart muscle were confirmed by suitably marked histological sections and the coronary arteries further studied by dissection along the easily visible injection mass. Finally, in a number of hearts injections were given in only one coronary artery or a branch in order to define better its distribution.

By this method there was available for each heart the roentgenogram of the uncut specimen and of the slices (Fig. 1), which could be superimposed on the outline drawings of the gross slices. Thus the pattern of myocardial involvement could be related in three dimensions to the coronary arteries.

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and Wartman, W. B.: Myocardial Infarction, Brit. Heart J. **6**:115, 1944. (*g*) Lowe, T. E., and Love, E. R.: Cardiac Aneurysms: A Mechanical Analysis of Their Formation, Australian J. Exper. Biol. & M. Sc. **26**:497, 1948. (*h*) Wartman, W. B., and Souders, J. C.: Localization of Myocardial Infarcts with Respect to the Muscle Bundles of the Heart, Arch. Path. **50**:329, 1950.

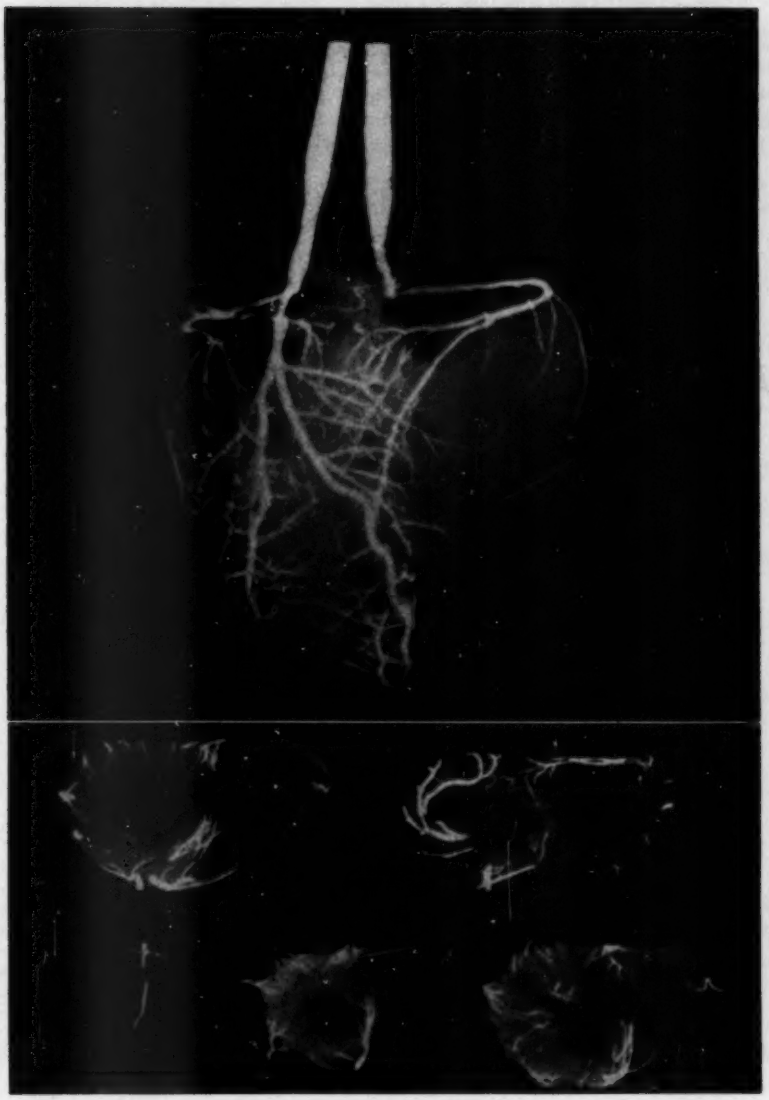

Fig. 1.—Roentgenograms of the entire injected heart and of the serial slices. Left coronary artery and left ventricle on the left.

A note is in order regarding the use of a barium mixture as a radiopaque medium. Its chief advantage is simplicity, since the mixture is easily made from commercially available barium, and the injection is carried out at room temperature, thus avoiding the apparatus and manipulation required with lead-agar and other mixtures which solidify at room temperature. It satisfies the criteria of being uninjurious to tissues, of having sufficient radiopacity, and of penetrating into the smallest arterioles but not the capillaries. Its viscosity is nearer to that of blood than the viscosity of other mixtures (barium mixure : blood = 1.5:1, lead-agar [at 45 C.] : blood = 3:1, barium-gelatin : blood = 18:1), and it lends itself easily to the addition of water-soluble dyes. Finally, after fixation the barium suspension does not leak out of the cut slices, as might have been expected, due to the hardening effect of the formalin.

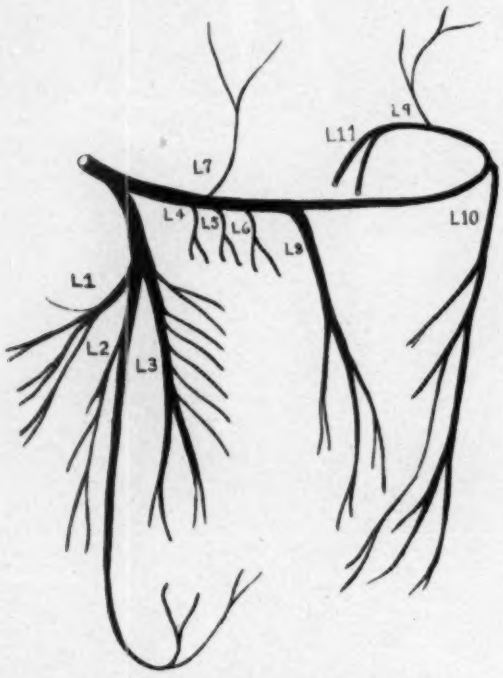


Fig. 2.—Diagram of the left coronary artery and branches. L1, left septal; L2, left anterior descending; L3, left anterior ventricular; L4, L5, L6, left adipose; L7, left anterior auricular; L8, left obtuse marginal; L9, left posterior auricular; L10, L11, left posterior ventricular in cases of left artery predominance, otherwise correspond to R11 and R12 in Figure 3.

## RESULTS

*Normal Anatomy of Coronary Arteries.*—The original diagram of Spalteholz [3] remains the definitive map of the coronary circulation. The major deficiency of this diagram, as pointed out by Schlesinger,[1] is its failure to define the blood supply of the myocardium of the interventricular septum. Schlesinger's roentgenograms clearly show that the septum is supplied by both the right and the left coronary artery,

3. Spalteholz, K. W.: Die Arterien der Herzwand: Anatomical Untersuchungen an Menschen- und Tierherzen, nebst Erörterung der Voraussetzungen für die Herzstellung eines Kollateralkreislaufes, Leipzig, S. Hirzel, 1924.

but he states [1] that "one or more large branches of the left descending coronary artery regularly penetrate this structure. It also ordinarily receives a large number of smaller branches from its posterior border. These branches of posterior origin vary in their source, depending on which vessels traverse the posterior interventricular groove. These may be branches either of the right or left coronary artery, or both."

Figures 2, 3, and 4 illustrate a constant finding in our series of hearts, showing that the septal myocardium has a constant and predictable blood supply. The anterior portion is supplied chiefly by a constant septal branch (*L1*) of the left coronary artery. This artery in two-thirds of our hearts comes off as a separate branch

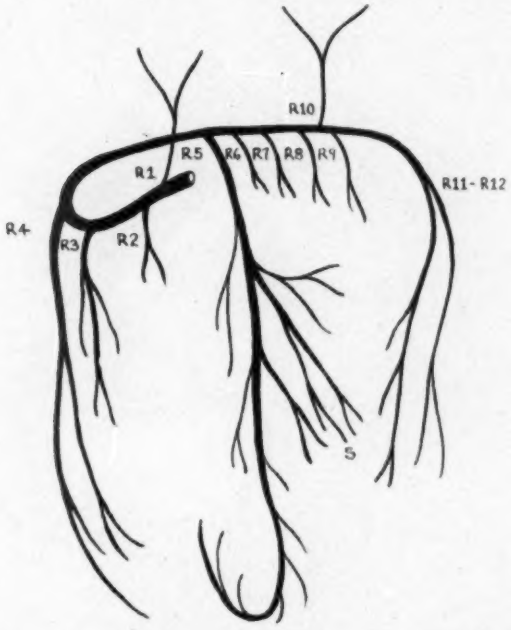


Fig. 3.—Diagram of the right coronary artery and branches. R1, right anterior auricular; R2, right anterior adipose; R3, right anterior ventricular; R4, right acute marginal; R5, right posterior ventricular; R6, R7, R8, R9, right posterior adipose; R10, right posterior auricular; R11, R12, accessory left posterior ventricular, variable, occurs as illustrated in right artery predominance; S, posterior septal branches.

distinct from the left anterior descending artery (*L2*), while in one-third it branches off from *L2* near its origin. The posterior portion of the septum is regularly supplied by septal branches of the right coronary artery branch, designated as *R5* in our diagrams. We have found no exceptions to this. In 80% of our series of hearts the arteries coming down the posterior interventricular groove are branches of the right coronary artery, and, when these branches come from the left coronary artery, they are not involved in supplying the posterior septum, since this supply is regularly from *R5*. There is occasionally a minor contribution from branches of *R4*.

The septal blood supply is shown in Figure 4, which also illustrates the contribution of various coronary branches at different levels. It should be noted that the branches designated as *L10* and *L11,* and branches *R11* and *R12* are the same, the number depending on which coronary artery they originate from. Contrary to Spalteholz [3] and Saphir [4] and in agreement with Schlesinger,[1] we have not found any true anastomoses between the terminal portions of the two coronary arteries in this posterior area.

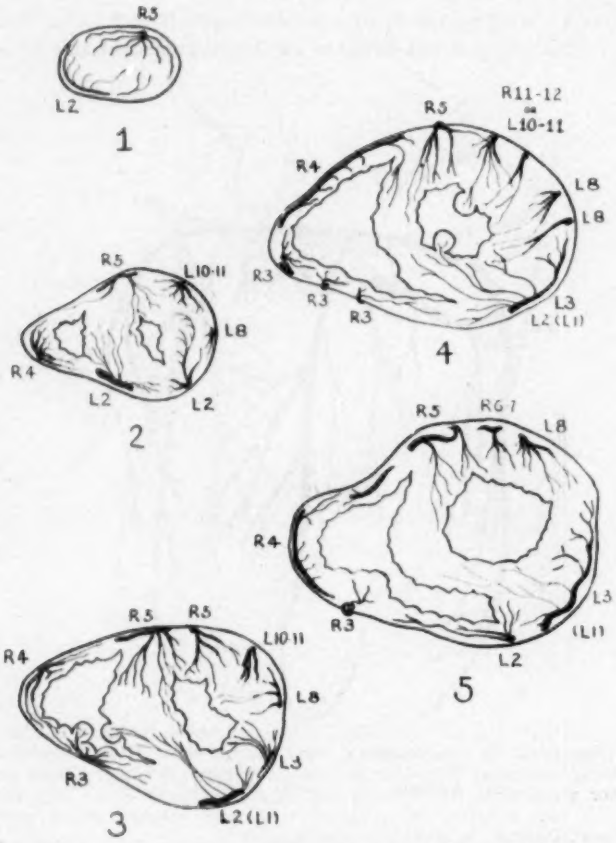


Fig. 4.—The blood supply of the myocardium as seen in cross sections at various levels. Anterior surface of heart face down, left ventricle on the right. Slice No. 1 is the tip; progressing toward the base, slice No. 5. Variations depend chiefly on the degree of differential ventricular hypertrophy and on the distribution of the terminal branches of the two main arteries.

The dependence of the posterior portion of the septum on the right coronary artery for its blood supply is well illustrated in the following case:

4. Saphir, O.; Priest, W. S.; Hamburger, W. W., and Katz, L. N.: Coronary Arteriosclerosis, Coronary Thrombosis and the Resulting Myocardial Changes, Am. Heart J. **10**:567, 1935.

F. K., a 77-year-old woman, died 12 hours after admission to the hospital. Three days previously she had noted sudden onset of left arm pain, abdominal cramps, and vomiting. Chest pain developed later that day and continued with increasing frequency and severity. She was admitted in acute distress, showing shock, cyanosis, and evidence of left cardiac failure, from which she did not recover. An electrocardiogram before death indicated "posterior myocardial infarction."

The cardiac findings at autopsy were (*a*) 100 cc. of bloody fluid in the pericardial sac; (*b*) subepicardial hemorrhage posteriorly near the base; (*c*) heart weight 500 gm.; (*d*) roentgenographic evidence (Fig. 5) of nonfilling of the entire right coronary artery, which on dissection and histologic examination was found to be occluded by a fresh thrombus; (*e*) roentgenographic

Fig. 5.—Patient F. K., showing failure of injection medium to fill the right coronary artery.

evidence of the avascular area in which the infarction occurred (Fig. 6 *A*), and (*f*) infarction in the posterior interventricular septum, with rupture of the septum (Fig. 6 *B*).

*Blood Supply of Myocardium, with Reference to Muscle Bundles.*—The structure of the heart with respect to muscle bundles has occupied the attention of anatomists for many years.[5] Robb and Robb [2a,b] and Lowe [6] have emphasized these

5. Lower, R., quoted by Walmsley, T.: The Heart, in Quain, J.: Elements of Anatomy, Ed. 11, New York, Longmans, Green & Co., 1929, Vol. 4, Pt. 3, p. 73. Mall, F. P.: On the Muscular Architecture of the Ventricles of the Human Heart, Am. J. Anat. **11**:211, 1911. Flett, R. L.: The Musculature of the Heart, with Its Application to Physiology, and a Note on Heart Rupture, J. Anat **62**:439, 1928. Robb, J. S.: Historical Survey of Studies of

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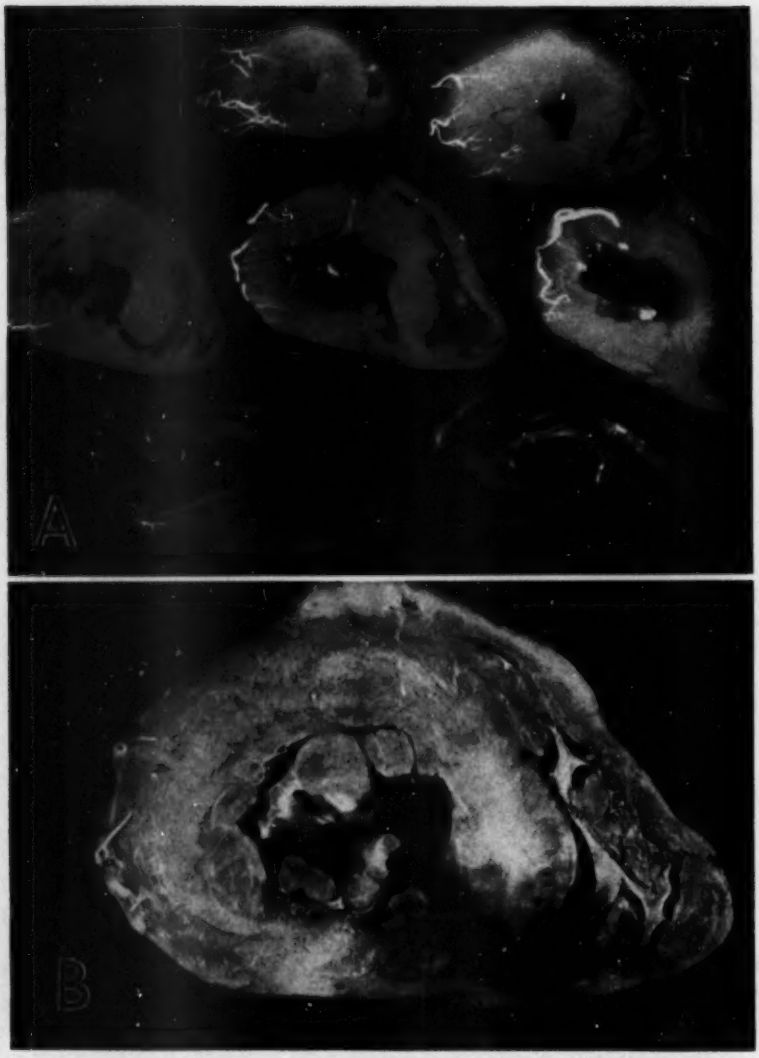


Fig. 6.—Patient F. K., *A*, roentgenogram of heart slice showing nonvascularity and the area of interventricular rupture. *B*, photograph of heart slice showing the extent of infarction and location of the rupture. A thrombosed branch of the right coronary artery is also shown.

concepts. This anatomical observation acquired importance when Robb and Robb,[2a,b] Lowe,[7] and more recently Wartman and Sounders [2h] presented data indicating that myocardial scars and infarcts have a muscle bundle distribution. From the clinical viewpoint, Levine and Ford [8] have predicted a typical electrocardiographic pattern when the subendocardial bundles are infarcted.

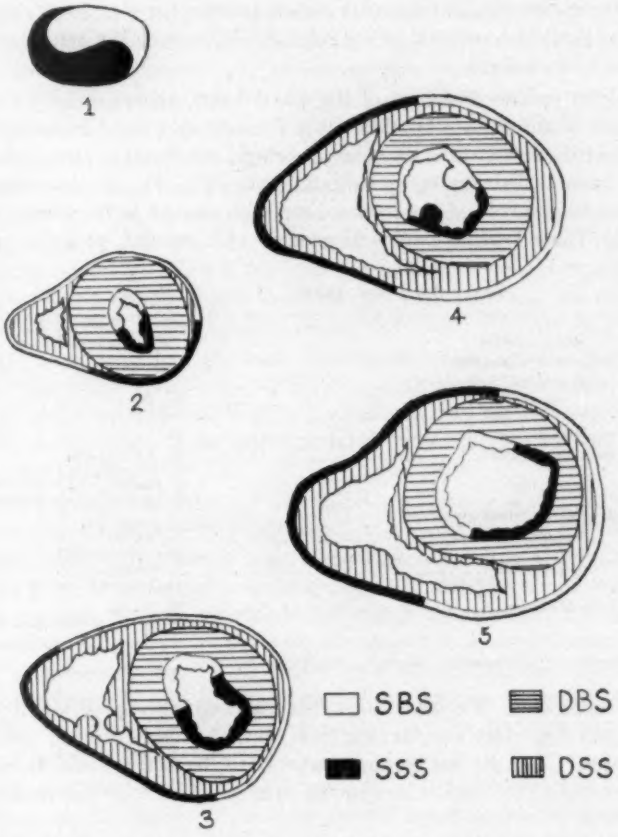


Fig. 7.—The cross sectional anatomy of the usual management of the four major cardiac muscle bundles. Arrangement of slices as in Figure 4. SSS, superficial sinospiral muscle; SBS, superficial bulbospiral muscle; DSS, deep sinospiral muscle; DBS, deep bulbospiral muscle. Variations are usually minor, except for the deep bulbospiral muscle, which in some cases does not extend as far towards the tip as shown in the diagrams.

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Ventricular Structure, M. Woman's J. **41**:203, 1934. Robb, J. S., and Robb, R. C.: Progressive Muscular Anemia in Heart of Dog, Proc. Soc. Exp. Biol. & Med. **37**:187, 1937. Shaner, R. F.: On the Muscular Architecture of the Vertebrate Ventricle, J. Anat. **58**:59, 1923.

6. Footnote 2 *c*, *d*, and *e*.

7. Footnote 2 *c*, *d*, *e*, *f*, and *g*.

8. Levine, H. D., and Ford, R. V.: Subendocardial Infarction: A Report of 6 Cases and Critical Survey of the Literature, Circulation **1**:246, 1950.

If there is a causal relationship between occlusion of a given artery and necrosis of a particular bundle, then it should be expected that for each bundle there is a definite, individual, and predictable blood supply. Robb and Robb [2a] attacked this problem by dissection of the arterial branches and bundles and presented a diagram showing a characteristic blood supply for the various bundles, although the diagram was modified by numerous qualifications in the text. Wartman and Souders [2b] concur in the distribution of infarcts in muscle bundles but were unable by dissection methods to clarify the question of the relationship between the artery occluded and the location of the infarct.

By making outline drawings of the sliced heart, representing the extent and conformation of the muscle bundles (Fig. 7), and then superimposing the roentgenograms of the same slices on these drawings, we found a remarkably constant pattern of arterial distribution at various levels (Fig. 4). As determined by this method the blood supply of the myocardium with respect to the four chief bundles is shown in Table 1. This agrees in general with the data of Robb and Robb,[2a]

TABLE 1.—*Blood Supply of Muscle Bundles*

| Muscle Bundle | Coronary Branch * |
|---|---|
| Superficial bulbospiral | |
| Superficial portion | |
| Lower half | $R_4$, $R_5$ |
| Upper half | $L_2$, $L_3$, $L_5$ |
| Deep portion | $R_5$ |
| Deep bulbospiral | $R_3$, $L_5$ (mostly)<br>$L_3$ (occasionally)<br>$L_1$ (anterior interventricular septum)<br>$R_5$ (posterior interventricular septum) |
| Superficial sinospiral | |
| Superficial portion | $L_3$, $R_3$, $R_4$<br>$R_5$ (occasionally) |
| Deep portion | $L_3$ (mostly)<br>$L_5$, $L_5$ (near base) |
| Deep sinospiral | $R_3$, $R_4$, $R_5$<br>$L_1$, $L_5$ (interventricular septum) |

*For explanation of abbreviations, see legend for Figure 3.

with some exceptions. We have found that the deep bulbospiral muscle receives a very large and important contribution from both the right coronary artery (in the posterior aspect) and the left coronary artery (in the anterolateral aspect), rather than being supplied by smaller descending branches of the left circumflex, as indicated by Robb.

However, it seems obvious that each bundle does not possess its own blood supply. The distribution is generally regional, so that the blood supply of the bundle varies in different portions, being generally dependent on the artery nearest it at a given level.

Table 2 summarizes the findings in 30 out of 81 hearts studied. These cases represent all hearts in which areas of recent necrosis or definitive patches of old scarring could be identified. In each case where the occluded vessel could be identified, we found excellent agreement with the expected area of damage as predicted from Figure 4. However, although in a few cases a simple muscle bundle distribution is obvious, in most cases there is either a partial involvement of one bundle or there is considerable overlapping between adjacent bundles. These data agree

essentially with those of Wartman and Souders,[2h] although we prefer to interpret these findings as indicating that muscle bundle involvement is more apparent than real, as might be predicted from our diagrams of their blood supply.

*Effect of Coronary Anastomoses on Location and Extent of Myocardial Damage.*—It has been pointed out by Porter,[9] Wiggers,[10] and Blumgart [11] that, although fine communications of less than 40 $\mu$ regularly exist between the right and left coronary arteries, these are not of any functional significance in the sense of furnishing an anastomotic circulation. An anastomotic circulation may then be said to exist when these anastomotic precapillary vessels enlarge to a functional caliber. We have determined this in our series by (*a*) actual continuity on the roentgenograms and by dissection and (*b*) retrograde flow of colored injection material.

Admittedly, the demonstration of anastomoses in a postmortem specimen does not prove that these were functional during life or that other anastomoses may not have been present. Nevertheless, since no other methods have been devised in a study of the degree of collateral circulation in the living human heart, the only data of which we may avail ourselves at this time are those from postmortem studies.

Two types of anastomoses were recorded in our observation, the first between branches of the right and left coronary artery, the second between branches of the same artery. Our concept of the main anastomotic zones between right and left coronary arteries follows closely that of Schlesinger,[1] i. e., (*a*) in the anterior portion of the right ventricle to the right of and parallel to the anterior interventricular groove, (*b*) in the region of the posterior interventricular groove, and (*c*) in the interventricular septum.

In correlating pathologic findings with the clinical history of each patient, we are in agreement with Blumgart [11] that functional anastomosis between coronary arteries is established only in response to need, regardless of the age of the patient. For example, we have demonstrated a striking degree of anastomosis in some children and no anastomosis in some patients in the eighth decade. The establishment of such an anastomotic circulation must be considered as an ever changing process paralleling progressive coronary disease or other myocardial need, with constantly changing hemodynamics, which sometimes make it difficult to predict the localization of ischemia. When narrowing occurs more rapidly than functionalization of anastomoses, ischemia will result.

From the data presented in Table 3 two main correlations between clinical data and the presence and degree of coronary arterial anastomoses have evolved: (*a*) the development of collateral circulation in response to increased myocardial need and (*b*) a roughly proportional relationship between myocardial tolerance of ischemia-producing factors and the presence and degree of anastomoses.

9. Porter, W. T.: Circulation: The Nutrition of the Heart, in Howell, W. H.: An American Textbook of Physiology, Ed. 2, Philadelphia, W. B. Saunders Company, 1901, Vol. 1, p. 179.

10. Wiggers, C. J.: The Physiology of the Coronary Circulation, in Levy, R. L.: Diseases of the Coronary Arteries and Cardiac Pain, New York, the Macmillan Company, 1936.

11. Blumgart, H. L.; Schlesinger, M. J., and Davis, D.: Studies on the Relation of Clinical Manifestations of Angina Pectoris, Coronary Thrombosis, and Myocardial Infarction to the Pathologic Findings with Particular Reference to the Significance of the Collateral Circulation, Am. Heart J. **19**:1, 1940.

TABLE 2.—*Summary of Findings in Thirty Hearts Showing Recent or Old Infarcts*

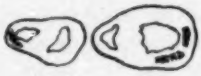
| Case | Heart Wt., Gm. | Coronary Pathology | Bundle Involvement* | Myocardial Lesions | Diagram ■ Necrosis ■ Scarring |
|---|---|---|---|---|---|
| 1 | 340 | Sclerosis ++++<br>Anastomoses +<br>Narrowing L main<br>Thrombosis R main | SBS, partial<br>SSS, partial<br>DBS, partial<br>DSS, partial | Rupture; acute necrosis | <br>Slice 4 |
| 2 | 400 | Sclerosis ++<br>Anastomoses +++<br>Thrombosis L main | SBS, deep, entire<br>DBS, partial | Necrosis | <br>Slice 2 |
| 3 | 750 | Sclerosis ++++<br>Anastomoses +++<br>Thrombosis $L_3$ | DBS, partial | Necrosis | <br>Slice 4 |
| 4 | 540 | Sclerosis ++++<br>Anastomoses O<br>Thrombosis $L_1$, $L_2$, $L_3$ | SSS, partial<br>DSS, partial<br>DBS, partial<br>SBS, partial | Scarring | <br>Slice 2 |
| 5 | 640 | Sclerosis ++++<br>Anastomoses ++++<br>Narrowing, patchy, particularly right coronary orifice | DSS, partial<br>DBS, partial | Scarring |  <br>Slice 3 Slice 5 |
| 6 | 600 | Sclerosis ++++<br>Anastomoses ++++<br>Narrowing, patchy | SSS, entire<br>DBS, partial | Scarring | <br>Slice 1 |

TABLE 2.—*Summary of Findings in Thirty Hearts Showing Recent or Old Infarcts*—Continued

| | | | | | |
|---|---|---|---|---|---|
| 7 | 520 | Sclerosis +++<br>Anastomoses +<br>Thrombosis L main | SBS, entire, deep<br>SSS, entire, deep<br>DBS, partial | Necrosis; patchy scarring | <br>Slice 4 |
| 8 | 320 | Sclerosis +<br>Anastomoses ++<br>Thrombosis L main | SBS, entire, superficial<br>SSS, entire, deep | Necrosis | <br>Slice 4 |
| 9 | 450 | Sclerosis +<br>Anastomoses +<br>Thrombosis L 10 | | Necrosis | <br>Slice 2 |
| 10 | 380 | Sclerosis ++++<br>Anastomoses ++<br>Thrombosis R main distally | DSS, partial<br>SSS, partial<br>DBS, partial | Necrosis; scarring | <br>Slice 3 |
| | 490 | Sclerosis ++<br>Anastomoses + | SBS, partial<br>DBS, partial<br>DSS, partial | Necrosis, with mural thrombosis; scarring | <br>Slice 2 |
| 12 | 450 | Sclerosis ++++<br>Anastomoses + | SSS, partial | Scarring | <br>Slice 3 |

TABLE 2.—*Summary of Findings in Thirty Hearts Showing Recent or Old Infarcts*—Continued

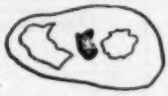
| | | | | | |
|---|---|---|---|---|---|
| 13 | 350 | Sclerosis O<br>Thrombosis $R_5$ | DBS, partial | Necrosis; scarring |  <br>Slice 5 |
| 14 | 500 | Sclerosis +++<br>Anastomoses +++<br>Narrowing, patchy | DBS, partial<br>SSS, partial<br>DSS, partial | Necrosis; scarring | <br>Slice 1 |
| 15 | 520 | Sclerosis ++++<br>Anastomoses ++ | SSS, entire, deep<br>SBS, partial<br>DSS, partial<br>DBS, partial | Necrosis, with mural thrombosis | <br>Slice 3 |
| 16 | 620 | Sclerosis +<br>Anastomoses ++ | DBS, partial | Scarring | <br>Slice 3 |
| 17 | 500 | Sclerosis +<br>Anastomoses +<br>Thrombosis R main | DBS, partial<br>DSS, partial<br>SSS, partial<br>SBS, partial | Necrosis, with rupture of interventricular septum | <br>Slice 4 |
| 18 | 375 | Sclerosis ++++<br>Anastomoses +++<br>Thrombosis $L_{10'}$, $R_5$ | DBS, partial<br>SBS, entire, deep<br>SSS, entire, deep | Necrosis; scarring | <br>Slice 3 |

TABLE 2.—*Summary of Findings in Thirty Hearts Showing Recent or Old Infarcts*—Continued

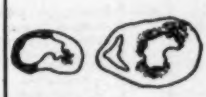
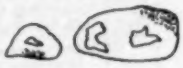
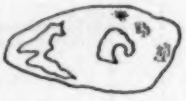
| | | | | | |
|---|---|---|---|---|---|
| 19 | 780 | Sclerosis ++++<br>Anastomoses ++++<br>Narrowing, patchy, most severe in $L_2$ and $L_3$ | DBS, partial<br>SBS, partial<br>DSS, partial<br>SBS, partial | Scarring | <br>Slice 5 |
| 20 | 400 | Sclerosis +++<br>Anastomoses +<br>Occlusion of L main by atheromatous material<br>Extreme patchy narrowing, scattered | SSS, partial<br>SBS, partial<br>DSS, partial<br>DBS, partial | Scarring; aneurysm of left ventricular wall |  <br>Slice 1 Slice 5 |
| 21 | 750 | Sclerosis ++++<br>Anastomoses +++<br>Narrowing marked at $L_2$, $R_3$, $R_5$<br>Old organized thrombus in $L_2$ | SSS, partial<br>SBS, partial<br>DSS, partial<br>DBS, partial | Scarring; aneurysm of left ventricular wall anteriorly | <br>Slice 4 |
| 22 | 450 | Sclerosis ++<br>Anastomoses ++++ | SSS, partial | Scarring of papillary muscle only | <br>Slice 3 |
| 23 | 420 | Sclerosis ++<br>Anastomoses + | SBS, partial | Scarring of papillary muscle only | <br>Slice 3 |
| 24 | 420 | Sclerosis ++<br>Anastomoses ++++<br>Narrowing of L main and $L_2$ | SSS, partial<br>SBS, partial | Scarring of papillary muscle only | <br>Slice 4 |

TABLE 2.—*Summary of Findings in Thirty Hearts Showing Recent or Old Infarcts*—Continued

| | | | | | |
|---|---|---|---|---|---|
| 25 | 550 | Sclerosis ++++<br>Thrombosis L main and L circumflex<br>Anastomoses +++ | SSS, partial<br>SBS, partial<br>DSS, partial<br>DBS, partial | Necrosis; laminar scarring; mural thrombosis; aneurysm of left ventricle with calcification | <br>Slice 2 Slice 5 |
| 26 | 600 | Sclerosis ++++<br>Anastomoses ++++<br>Narrowing right ostium and R main<br>Cholesterol occlusion L circumflex<br>Narrowing $L_2$ | SSS, partial<br>SBS, partial<br>DSS, partial<br>DBS, partial | Left ventricular aneurysm near apex; scarring; necrosis | <br>Slice 2 |
| 27 | 470 | Sclerosis +++<br>Anastomoses ++++<br>Narrowing, all branches | SSS, partial<br>SBS, partial | Diffuse old scars |  <br>Slice 2 Slice 3 |
| 28 | 340 | Sclerosis +++<br>Anastomoses +++<br>Narrowing R main, L main, and branches | SSS, partial<br>SBS, partial<br>DSS, partial<br>DBS, partial | Old scars | <br>Slice 1 Slice 3 |
| 29 | 270 | Sclerosis ++<br>Anastomoses ++++<br>Narrowing, L main and branches | SBS, partial<br>DSS, partial<br>DBS, partial | Old scars |  <br>Slice 3 |
| 30 | 470 | Sclerosis +++<br>Anastomoses ++++<br>Narrowing L main and branches<br>Narrowing R main and $R_3$ | SSS, partial<br>SBS, partial<br>DSS, partial<br>DBS, partial | Old scars; anterior left ventricle aneurysm | <br>Slice 3 |

* For explanation of abbreviations, see legend for Figure 7.

TABLE 3.—*Relationship Between Anastomotic Circulation, Myocardial Damage, and Survival*

| Anastomoses | No. of Cases | Coronary Lesions | Myocardial Pathology | Clinical Picture | Survival After Acute Coronary Symptoms |
|---|---|---|---|---|---|
| 0 | 1 | Thrombosis | Wt. 550 gm. | Obesity; B.P. 160/100 | Less than 24 hr. |
| | 1 | Narrowing and thrombosis | Scar; wt. 540 gm. | Obesity; angina; fibrillation | Less than 24 hr. |
| | 1 | Narrowing | Old pericarditis | Fibrillation, left ventricular strain | |
| | 1 | Congenital absence of left coronary | Congenital malformation | Cyanosis; dyspnea, 20 hr. postnatally | Less than 48 hr. |
| | 7 | None | None | Dissecting aneurysm; subarachnoid hemorrhage; diabetes; burn toxemia; prematurity complication; meningitis; accidental injuries | |
| Total | 11 | | | | |
| + | 1 | Narrowing and thrombosis | Infarction and scar | Chronic orthopnea; B.P. 150/110; obesity; angina; ankle edema terminally | 5 days |
| | 1 | Narrowing and thrombosis | Infarction and rupture | Obesity; coronary thrombosis 3 yr. previous | 8 days |
| | 1 | Narrowing | Infarction and scar | B.P. 170/90; angina and arrhythmia few yr. | No acute coronary symptoms; died of cerebrovascular accident |
| | 1 | Narrowing; rt. preponderance | Infarction | Angina 2 days; fibrillation | 3 days |
| | 1 | Uniformly small with sclerosis | Infarction; valvular disease; wt. 450 g m. | Obesity; B.P. 260/130; congestive failure; arrhythmia | No acute coronary symptoms; died of emboli |
| | 1 | Narrowing to occlusion | Scar with aneurysm | Angina; occlusion syndrome 2 yr. previous; died of cerebral vascular accident | 2 yr. |
| | 1 | Narrowing | Scar | Fibrillation, left ventricle strain; diabetes; congestive failure | 7 days after onset acute failure |
| | 1 | Narrowing | Scar | Treated syphilis; obesity; B.P. 180/88; congestive failure with pneumonia; heart attack 1945 | 7 yr. |
| | 6 | Narrowing | Hypertrophy of 4 | Angina; obesity; hemorrhage; hypertension; diabetes; valvular disease; carcinomas; infections | |
| | 1 | Uniformly small with sclerosis | Extremely flabby; wt. 400 gm. | Carcinoma of bladder with pyelonephritis | |
| | 5 | None | Hypertrophy 1; diffuse fibrosis 1 | Hypertension; valvular disease; bronchial asthma; congestive failure; thromboembolic phenomena; nephrosis; carcinoma | |
| Total | 20 | | | | |
| ++ | 1 | Narrowing and thrombosis | Infarction; scar; pericarditis | B.P. 190 systolic; diabetes; blood loss; postoperative cardiac failure | 17 days |
| | 1 | Narrowing and thrombosis | Infarction with septal rupture | Obesity; mitral stenosis; shock 3 days after occlusion syndrome | 4 days |
| | 1 | Narrowing | Infarction and scars | Coronary episodes 10 and 20 yr. previous; obesity; mild diabetes; occlusion syndrome | 4 wk. |
| | 1 | Narrowing | Infarctions and left ventricle mural thrombosis | Exertional dyspnea and flatulence; occlusion syndrome; refusal of medical care | 10 days |
| | 1 | Narrowing and thrombosis | Infarction and scar; mural thrombosis 2 ventricles | Occlusion syndrome; thromboembolic phenomena | 25 days |
| | 1 | Thrombosis | Infarction | Previous anginal attacks; occlusion syndrome; vascular collapse | Less than 24 hr. |
| | 1 | Narrowing | Scar and diffuse fibrosis | Pulmonary infaret and bronchopneumonia | |
| | 1 | Uniformly small but extensive | Fragmentation fibers; wt. 360 gm. | Primary pulmonary thrombi with infarction; died in 17 days | |
| | 4 | Narrowing | Diffuse fibrosis, 3 | Diabetes; cachexia; carcinoma; sprue; valvular disease | |
| | 2 | None | Diffuse fibrosis, 1 | Treated syphilis; mitral disease | |
| Total | 14 | | | | |
| +++ | 1 | Narrowing and thrombosis | Infarction; scars; left ventricle aneurysm | Hypertension (B.P. 170/100) and angina 19 yr. | 2 mo., 1 and 3 yr. |
| | 1 | None | Infarction and scar | Occlusion syndrome; obesity; carcinoma of colon | 9 wk. |

TABLE 3.—*Relationship Between Anastomotic Circulation, Myocardial Damage, and Survival*—Continued

| Anastomoses | No. of Cases | Coronary Lesions | Myocardial Pathology | Clinical Picture | Survival After Acute Coronary Symptoms |
|---|---|---|---|---|---|
| | 1 | Narrowing | Infarction; scar; diffuse fibrosis; hypertrophy (750 gm.) | Digitalis for 2 yr. previous; occlusion syndrome; arrhythmia; congestive failure | 7 days |
| F | 1 | Thrombosis | Infarctions | Angina 1 mo.; occlusion syndrome | 24 hr. |
| | 1 | Narrowing and mushy clot | Infarction | Occlusion syndrome 2 days postoperative | 4 days |
| | 1 | Organized thrombus; narrowing | Scars and left ventricle aneurysm; old pericarditis | Cardiac treatment 5 yr. previous; peripheral edema 6 mo. | Sudden death |
| | 1 | Narrowing | Scattered small scars | Syphilis with aortic aneurysm; ruptured peptic ulcer after open reduction patella | |
| | 1 | Narrowing | Early anoxic changes; diffuse fibrosis | B.P. 220/120; died of Friedländer's bacillus in less than 24 hr. | |
| | 1 | Uniformly small with slight narrowing points | Dilatation; flabbiness; diffuse fibrosis; wt. 840 gm. | Congestive failure; fibrillation; bundle branch block; pneumonia | |
| | 11 | Narrowing, 3 | Scars, 3; diffuse fibrosis, 4 | Anemia; hypertension; valvular disease; leukemia; diabetes; melanoma; atrioventricularis communis; rheumatic fever; carcinoma; obesity; uremia; asthma; hemorrhage; injuries; infections | |
| Total | 20 | | | | |
| ++++ | 1 | Narrowing; cholesterol occlusion | Infarction; scars with left ventricle aneurysm | Obesity; diabetes; congestive failure; bronchopneumonia; B. P. 120/70 | |
| | 9 | Narrowing | Scars, 5 | 1 hypertension; angina; congestive failure; gangrenous peritonitis | |
| | | | | 1 cardiac asthma; sudden death 6 days postoperative | |
| | | | | 1 obesity; B.P. 115/60; thrombocytopenia, with fatal intracranial hemorrhage | |
| | | | | 1 metastatic carcinoma with ascites, 8 mo. | |
| | | | Left ventricle aneurysm | 1 liver cirrhosis; chronic and acute pancreatitis; auricular fibrillation | |
| | | 1 right coronary preponderance | ..... | Hypertension; congestive failure; accidental injuries | |
| | | | | 1 bronchial asthma; pneumonia; pulmonary infarction | |
| | | | | 1 agranulocytosis; anemia; pulmonary infarction; aortic stenosis | |
| | | | | 1 carcinoma and peritonitis | |
| | 4 | ..... | ..... | Rheumatic mitral and aortic disease | |
| | | | | 1 embolic cerebrovascular accident | |
| | | | | 1 multiple embolic phenomena; vascular collapse | |
| | | | | 1 subacute bacterial endocarditis | |
| | | | Scar, 1 | Congestive failure | |
| | 1 | ..... | ..... | Extreme obesity; B.P. 250/140; para 15; congestive failure, with anasarca | |
| | 1 | ..... | ..... | Hypocephaly; cachexia; bronchopneumonia (3 yr. old) | |
| Total | 16 | | | | |

Thus, the following considerations will be noted:

1. In the patients having no demonstrable anastomoses (0), 3 of 11 had arteriosclerotic coronary disease, and of these the 2 who developed acute coronary thrombosis died quickly.

2. In those patients having a few anastomoses (1 +), 15 of 20 had arteriosclerotic coronary disease, and 5 evidenced previous survival of myocardial infarction, 1 dying from a cerebrovascular accident two years after the acute coronary thrombosis. Of the five cases of recent infarction, two patients had acute thrombosis and died in five and eight days (one of ventricular rupture and acute cardiac tamponade); one patient had myocardial infarction due to arterial narrowing, without

demonstrable thrombosis, and in the other two patients myocardial infarction was discovered in the routine study of the heart and probably served only as a contributory factor in deaths chiefly due to cerebral and pulmonary vascular accidents.

3. In the patients with moderate anastomoses (2 +), 12 of 14 had arteriosclerotic coronary disease. Five evidenced previous survivals of infarction, one for as long as 20 years. Of the six patients who died of recent infarction (one with septal rupture), four had other serious diseases (diabetes, severe blood loss, mitral stenosis, thromboembolic phenomena); one refused to accept activity restrictions, and one died of thrombotic occlusion and vascular collapse, culminating a series of untreated anginal attacks.

4. In the patients with many anastomoses (3 +), 9 of 20 had arteriosclerotic coronary disease, and 8 evidenced previous survival of infarction. Of the six patients with acute infarction, one (a severe hypertensive) died of Friedländer's bacillus pneumonia in less than 24 hours; one had thrombotic coronary occlusion, culminating one month of severe untreated anginal attacks. Of the other four, one patient died postoperatively; one had carcinomatosis; one had intractable congestive failure and cardiac arrhythmia previous to the final acute coronary episode. The fourth had final thrombotic occlusion of all main coronary branches, culminating 19 years of hypertension and angina, having survived infarctions one and two years before death.

5. In those having very many anastomoses (4 +), 10 of 16 had coronary disease; 7 evidenced previous survival of infarctions, and 1 had coronary occlusion (by cholesterol deposits) and recent infarction. Other causes of death were cardiac valvular disease, bronchial asthma and pulmonary infarction, overwhelming septicemia, agranulocytosis, peritonitis, hypertensive cardiovascular disease, acute pancreatitis, carcinoma with severe ascites.

In the patients without coronary or myocardial pathology but with demonstrable anastomoses, such anastomoses result from the need caused by other disease states. Of especial interest, then, were our observations of the diseases which apparently increased myocardial demand sufficiently to stimulate coronary anastomotic functionalization. There were, roughly in order of importance in our series, (*a*) rheumatic valvular heart disease, (*b*) congenital cardiac malformations, (*c*) hypertension, (*d*) bronchial asthma, (*e*) obesity, and (*f*) anemias. It must also be noted that in some of our patients these same stimuli apparently failed to evoke any significant degree of coronary anastomosis.

Finally, it should be recorded, but without an obvious explanation at this time, that in four of the hearts studied the coronary arteries were uniformly of small caliber. Only one of these exhibited any coronary pathology, and this consisted of patchy small calcium deposits but with no marked narrowing on this basis. Three of these hearts were hypertrophied (400, 450, and 840 gm.), and actually arteries of larger than normal caliber might have been expected. We feel that we have ruled out technical difficulties, among which might be mentioned the factor of arterial spasm, which is considered by us and others [1] as of no importance as far as the success of the injection is concerned, but of course we cannot be absolutely sure. We have noted also the exclusive predilection of the arteriosclerotic lesions for the

proximal one-half or two-thirds of the arterial tree in the heart, the smaller distal segments and particularly the anastomotic vessels being generally soft and thin-walled and appearing normal histologically.

## SUMMARY

A method is described for the injection of coronary arteries with radiopaque material. The method is simple, rapid, requires no complicated apparatus, and yields the required information.

Its more general adoption as a part of every autopsy is urged, since it is believed that with only a little more work a great deal more information can be obtained than is available by routine dissection.

By using this method we have presented the normal anatomy of the coronary tree, particularly with respect to cross sections.

Correlation between vascular distribution and arrangement of myocardial muscle bundles shows that muscle bundles do not have an individual blood supply.

Scars and infarcts usually involve only a portion of several adjacent bundles, and the distribution is dependent not on the arrangement of the bundle but on the pattern of vascular distribution.

Anastomotic channels are formed only in response to need, whether it is coronary narrowing or increased myocardial need, and do not parallel old age as such.

The presence and degree of anastomoses play a very important role not only in the localization of myocardial ischemia but also in the prognosis following myocardial infarction.

Dr. J. M. Wickham and Sister M. Clarissima, M.T., gave their assistance in the preparation of the roentgenograms.

# INTRACAPILLARY PRECIPITATES PRODUCED IN RABBITS BY MEANS OF SODIUM POLYANETHOLSULFONATE

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INDERBITZIN,[1] in 1951, using the high molecular weight anticoagulant sodium polyanetholsulfonate (Liquoid), produced in the leucocytes of normal human blood in vitro and in those of rabbit blood in vivo changes which resembled morphologically the changes characterizing the "L. E. phenomenon" as originally described by Hargraves.[2] One of us (P. M. D.) has recently repeated Inderbitzin's observations and confirmed his essential findings and has noted in addition that a fine granular precipitate regularly forms when sodium polyanetholsulfonate is added in vitro to rabbit plasma. During the course of this work a rabbit died suddenly some hours after receiving a single injection of sodium polyanetholsulfonate intravenously. Postmortem examination disclosed intracapillary precipitates in the glomeruli which resembled to some extent the "wire-loop" lesions frequently seen in the kidneys of patients with disseminated lupus erythematosus, with similar precipitates also in the capillaries of the lungs and in those of other organs. In further studies intracapillary precipitates were regularly found in the renal glomeruli and in other organs of rabbits dying several hours after a single injection of sodium polyanetholsulfonate; whereas the kidneys of rabbits given repeated doses of sublethal amounts of the material had interesting lesions resulting presumably from occlusion of the renal blood vessels by the intracapillary precipitates.

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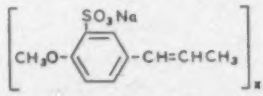
From the Departments of Pathology and Medicine, New York Hospital-Cornell Medical Center.

1. Inderbitzin, T.: Untersuchungen über die willkürliche Erzeugung von "Lupus-erythematodes-Zellen" im normalen Blut, Schweiz. med. Wchnschr. **81**:1298, 1951; Weitere Untersuchungen über die willkürliche Erzeugung von "Lupus-erythematodes-Zellen" im normalen Blut, ibid. **82**:561, 1952; The Experimental Production of Leukocytic Changes in Normal Blood Similar to LE-Cells and Morphologically Identical with LE-Cells, J. Invest. Dermat. **20**:67, 1953.

2. Hargraves, M. M.; Richmond, H., and Morton, R.: Presentation of Two Bone Marrow Elements: The "Tart" Cell and the "L.E." Cell, Proc. Staff. Meet., Mayo Clin. **23**:25, 1948; Production in Vitro of the L.E. Cell Phenomenon: Use of Normal Bone Marrow Elements and Blood Plasma from Patients with Acute Disseminated Lupus Erythematosus, ibid. **24**:234, 1949.

## MATERIALS AND METHODS

Sodium polyanetholsulfonate was generously supplied by Hoffman-La Roche, Inc. It is a polymer of sodium anetholsulfonate with the following formula:



It has been used as an anticoagulant in vitro and also as a stabilizer of colloidal solutions and as an adjuvant in blood cultures, but the mechanisms of its action are not known.[3]

For the purposes of the present work the white powdery substance, which is soluble in water and insoluble in alcohol, was made up in 1 or 2% solutions in distilled water and sterilized by filtration through a Seitz filter. Young adult market-bought rabbits of both sexes weighing between 2,000 and 3,500 gm. were given intravenous injections of the solutions, the amount given per injection ranging from 1 to 4 cc. None of the animals appeared to suffer any immediate ill effects from the injections, though small quantities of blood often oozed from the sites of venipuncture during approximately one hour. The animals given 10 or 15 mg. sodium polyanetholsulfonate per kilogram of body weight usually died abruptly some two and one-half to five and one-half hours after the injection. Prior to terminal convulsive seizures they manifested tachypnea with pink frothy sputum which was taken as evidence of acute pulmonary edema. Other animals were given repeated injection of sodium polyanetholsulfonate beginning with smaller amounts; these animals survived without signs of illness until the dosage levels were increased to a lethal level. One rabbit was given a single injection of sodium polyanetholsulfonate and was skin tested nine days later with 1 mg. of the material; there was no reaction within 24 hours. Some of the rabbits given repeated injections of sodium polyanetholsulfonate lost up to 10% of their body weight in the week preceding their death. Autopsies were held on all of the animals except two within an hour after death. Tissue blocks of heart, lungs, thymus, liver, spleen, pancreas, adrenals, kidneys, ovaries, testes, and lymph nodes were fixed in Zenker-formol solution, and hematoxylin and eosin stains of the paraffin-embedded preparations were examined, together with a few additional stains of the kidney sections. Tissue sections from many additional rabbits served for comparison. These included, in addition to normal controls, animals given injections of horse serum and chloramphenicol (Chloromycetin) and other animals with various experimental conditions.

## POSTMORTEM FINDINGS IN RABBITS DYING A FEW HOURS AFTER A SINGLE INTRAVENOUS INJECTION OF SODIUM POLYANETHOLSULFONATE

Table 1 provides a summary of the observations. From the Table it will be seen that intracapillary precipitates were regularly found in the glomeruli of rabbits dying a few hours after they had received a single injection of sodium polyanetholsulfonate intravenously and that similar precipitates were likewise conspicuous in the capillaries of the lungs, adrenals, and other viscera. The fact was especially noteworthy that the precipitates were absent from the capillaries of the brains of these seven rabbits, the findings as a whole suggesting that death had resulted in most, if not in all, instances from acute pulmonary congestion and edema due to occlusion of the capillaries of the lungs.

The kidneys of these seven rabbits on gross examination showed slight to moderate congestion, but otherwise they appeared normal. More than one-half of the

3. Fitch, C. P.; Bishop, L. M., and Kelley, M.D.: The Isolation of Brucella Abortus from the Blood Stream of Cattle, Proc. Soc. Exper. Biol. & Med. **34**:696, 1936. von Haebler, T., and Miles, A. A.: The Action of Sodium Polyanethol Sulphonate ("Liquoid") on Blood Cultures, J. Path. & Bact. **46**:245, 1938.

glomeruli in each kidney contained intracapillary precipitates, however, as microscopic studies showed, and these were conspicuous also in the afferent arterioles. The precipitates appeared as light reddish purple, occasionally metachromatic deposits in the sections stained with hematoxylin and eosin, as delicate reddish purple masses when stained with Schiff's periodic acid stain, as bright red deposits in preparations stained with Masson's trichrome stain, and as dark purple masses

TABLE 1.—*Postmortem Findings in Rabbits Dying After Single Intravenous Injection of Sodium Polyanetholsulfonate*

| Rabbit No. | Sex | Wt., Gm. | Total Dose, Mg. | Death, Hr. After Injection | Essential Postmortem Findings * |
|---|---|---|---|---|---|
| 1 | M | 2,000 | 20 | 5½ | Intracapillary precipitates forming casts in most of glomeruli † and alveolar septa. Numerous small capillary casts in adrenal cortex, perifollicular sinuses of spleen, liver sinusoids, alveolar spaces; conspicuous hemorrhages in adrenal cortices, numerous petechiae in other organs, with congestion |
| 2 | M | 3,650 | 55 | 2½ | Intracapillary precipitates forming casts in many glomeruli and alveolar septa,‡ a few in liver sinusoids and alveoli; conspicuous hemorrhages in adrenal cortices, numerous petechiae in other organs |
| 3 | F | 2,450 | 37 | 4 | Intracapillary precipitates in about 50% of glomeruli and alveolar septa; numerous small casts in perifollicular sinuses of spleen, few in capillaries of subcapsular cortical layer of adrenals and sinusoids of liver; small hemorrhages in adrenal cortices and other organs; small foci of bronchopneumonia |
| 4 | M | 3,000 | 45 | 7½ | Intracapillary precipitates forming casts in about 75% of glomeruli and lung septa, many in capillaries of subcapsular layer of adrenals, few in liver sinusoids; foci of hemorrhage and necrosis in medulla of kidneys; conspicuous hemorrhages in adrenal cortices, scattered petechiae in other organs; small foci of early bronchopneumonia |
| 5 | M | 2,950 | 44 | 3½ | Intracapillary precipitates forming casts in about 90% of glomeruli and alveolar septa, many in capillaries of perifollicular sinuses of spleen, few in liver sinusoids; conspicuous subendocardial hemorrhages, numerous petechiae in adrenal cortices and other organs |
| 6 | F | 2,400 | 36 | 2½ | Intracapillary precipitates forming casts in many glomeruli, few in alveolar septa; scattered small casts in capillaries of perifollicular sinuses of spleen and sinusoids of liver; small hemorrhages in adrenal cortices and other organs |
| 7 | F | 3,200 | 48 | 4 | Intracapillary precipitates forming casts in about 50% of glomeruli, many small casts in sinusoids of liver, frequently grouped around central veins; extensive occlusion of septal capillaries of lung by casts; conspicuous hemorrhages in lung and atria; scattered petechiae in other organs but not in adrenals |

* The changes in kidneys, lungs, and adrenals were bilateral in every instance.
† Figures 1 and 2.
‡ Figure 3.

when stained with Weigert's fibrin stain. As a rule the precipitates coated the insides of the capillaries, forming thin membranes or thick layers which varied in density from place to place and often narrowed the capillary lumen almost to the point of obliteration. Curving with the outlines of the glomerular capillaries, these deposits often resembled the "wire-loops" that characterize the altered glomerular tufts in disseminated lupus erythematosus (Figs. 1 and 2). In the most severely affected glomeruli large irregular precipitates filled and occluded several of the tufts over a distance of one-third to one-half the diameter of the glomerulus; whereas other deposits, which were smaller and more or less spherical, partially occluded

capillaries, though not infrequently they were separated from the capillary wall by a thin empty space. Some of the smaller more spherical deposits resembled the hematoxylin bodies likewise seen in disseminated lupus erythematosus, though they lacked the hyperchromatism of these bodies (Figs. 1 and 2). From the studies as a whole it was plain that the precipitates were generally within the lumina of the

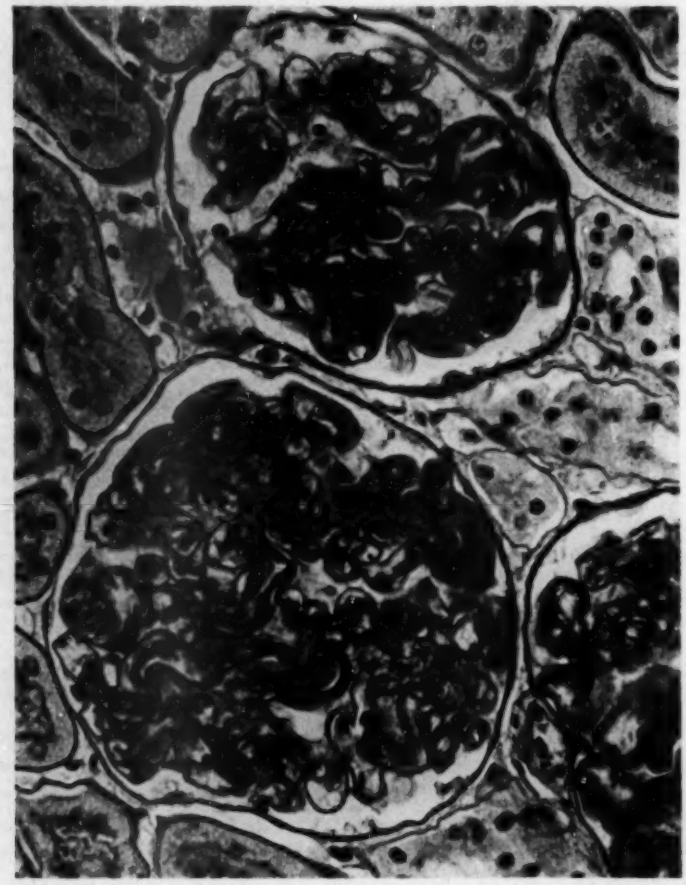


Fig. 1.—Kidney of rabbit that died five and one-half hours after the intravenous injection of sodium polyanetholsulfonate. Two glomeruli and part of another are shown. Precipitates are present in the capillary loops of all three glomeruli. These frequently produce a "wire-loop" effect. Occasional deposits occlude the capillaries; whereas others lie free in the lumen. The basement membranes are distinct. Schiff's periodic acid stain; × 450, slightly enlarged.

capillaries; yet more refined histological techniques will have to be employed before the relationships of the precipitates and basement membranes can be defined precisely.

On microscopic examination small precipitates very similar to those already described in the glomeruli were seen in the capillaries of other organs. Most con-

sistently involved, apart from the kidneys, were the lungs and liver. In the lungs the precipitates were often numerous in the septal capillaries (Fig. 3), and they were found occasionally in the pulmonary arterioles and arteries as well. In the sinusoids of the liver the small plugs of eosinophilic material were widely scattered, numbering less than one per high-power field as a rule. So too in the adrenals and

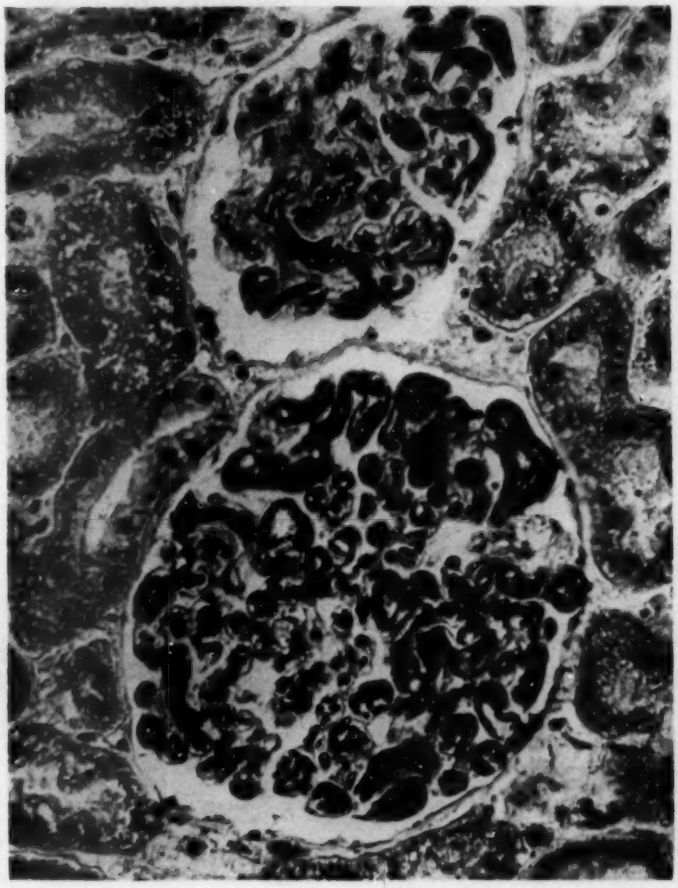


Fig. 2.—Same kidney as in Figure 1. The deposits are not homogeneous, and their support of endothelium and basement membranes is not clearly defined. A "wire-loop" is seen at the upper pole of the large glomerulus. Masson's trichrome stain; × 450.

spleen a few small deposits could be seen in the capillaries of the outer cortex and in the perifollicular sinuses, respectively.

All seven animals had small visceral hemorrhages of the magnitude and character usually encountered in agonal anoxia. Petechiae were commonly found in the adrenals, endocardium, epicardium, and thymus and on the pleural surfaces of the lungs. In addition, small interstitial and parenchymal hemorrhages were found in

the myocardium and in the lungs of four of the animals. Particularly striking was the presence of bilateral adrenocortical hemorrhages, which occurred in all but one of the animals. In all rabbits there was moderate to marked pulmonary edema and congestion of the viscera.

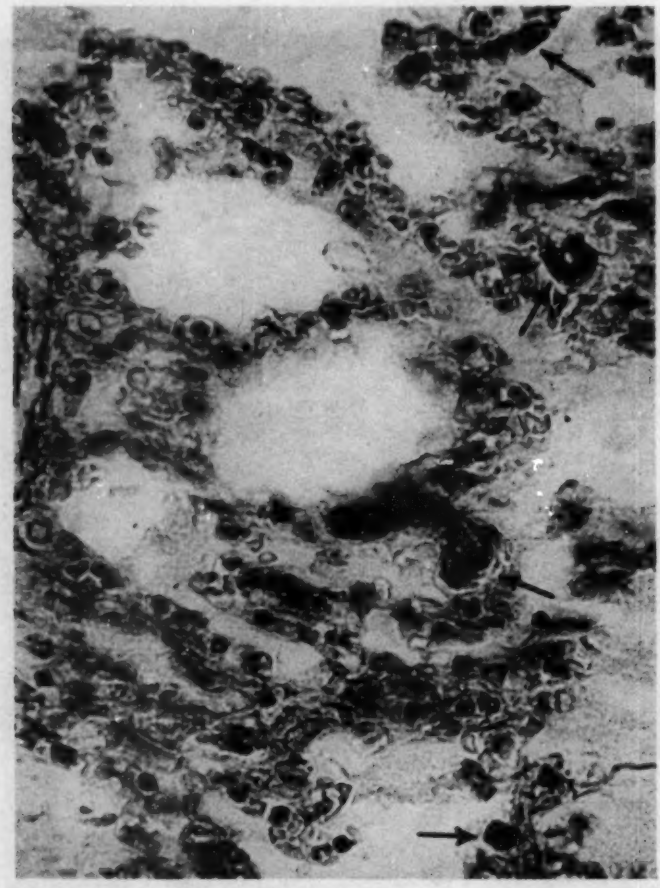


Fig. 3.—Lung of rabbit that died two and one-half hours after intravenous injection of sodium polyanetholsulfonate. In the center is a large irregular precipitate occluding a septal capillary (*arrow*). In the lower right-hand corner a round deposit appears to lie in the lumen of an alveolus, and in the upper right-hand corner several precipitates of different size and shape may be seen. There is also some pulmonary edema. Schiff's periodic acid stain; × 450, twice enlarged.

### ACUTE AND CHRONIC RENAL LESIONS AND OTHER CHANGES IN RABBITS GIVEN SODIUM POLYANETHOLSULFONATE

Five rabbits survived for some time after one or more injections of sodium polyanetholsulfonate and displayed secondary renal lesions and other changes on postmortem examination. A summary of the findings in these animals is given in

Table 2. In Rabbit 8, which was killed 10 days after a single intravenous injection of 10 mg. of sodium polyanetholsulfonate per kilogram of body weight, there were minute intracapillary precipitates in the glomerular and alveolar capillaries which

TABLE 2.—*Acute and Chronic Renal Lesions and Other Changes in Rabbits Given Sodium Polyanetholsulfonate*

| Rabbit No. | Sex | Wt., Gm. | Total Dose, Mg. | No. of Injections | Survival: Days After First Injection | Survival: Days After Last Injection | Essential Postmortem Findings * |
|---|---|---|---|---|---|---|---|
| 8 | F | 3,000 | 30 | 1 | 10 | 10† | Minute intracapillary precipitates in a few glomeruli and alveolar septa; many glomeruli with discrete adhesions between tufts and Bowman's capsule; no hemorrhages |
| 9 | M | 2,700 | 170 | 19 | 21 | 1½† | Intracapillary precipitates in a few glomeruli, thread-like deposits in a few septa of lungs; many glomeruli with adhesions between tufts and capsule; numerous macrophages laden with engulfed granular material present in spleen and occasional Kupffer cells |
| 10 | M | 2,250 | 205 | 22 | 24 | 1½ | Intracapillary precipitates in several glomeruli and a few septa and alveoli of lungs; occasional glomeruli with hemorrhagic necrosis, others with more or less extensive sclerosis of tufts; most cortical tubules necrotic; numerous macrophages filled with granular material present in sinuses of spleen and many Kupffer cells; necrosis with hemorrhages in adrenal cortices; many petechiae in other organs; small foci of interstitial pneumonia |
| 11 | M | 2,900 | 125 | 23 | 25 | ½ | Numerous adhesions between glomerular tufts and Bowman's capsule and slight intercapillary sclerosis; small intracapillary precipitates in alveolar septa; macrophages with ingested granular deposits diffusely scattered in the lumen of alveoli and sinuses of spleen; small foci of early bronchopneumonia |
| 12 | F | 2,800 | 422 | 40 | 45 | 3 | All glomeruli changed, most with varying stages of intercapillary sclerosis and adhesions between glomerular tufts and Bowman's capsule; several glomeruli with intracapillary precipitates, others with hemorrhagic necrosis; coagulation necrosis of many cortical tubules; many large macrophages filled with granular debris present in sinuses of spleen, a few in alveoli; numerous Kupffer cells of liver with similar material; early cirrhosis of liver, with slight fatty metamorphosis; meningo-encephalitis in brain, probably infectious ‡ |

* The renal changes were bilateral in every instance.
† Animal was killed.
‡ See text.

were similar to, but smaller than, those already described. In addition, there were small adhesions between the glomerular tufts and capsules. They were present also in various degrees in the other rabbits (Nos. 9, 10, 11, and 12) which had received repeated daily injections of sodium polyanetholsulfonate, doses of 1 to 2 mg. per kilogram of body weight per day being given at first and gradually increasing

amounts later until approximately 10 to 15 mg. per kilogram of body weight was injected each day. Rabbit 9, when killed 21 days after the first injection and 1½ days after the last of 19 injections, had precipitates in the capillaries of a few of the glomeruli and alveolar septa and many adhesions between tufts and capsule. In addition, there were in the spleen numerous macrophages containing engulfed eosinophilic granular material in their cytoplasm, and similar material was present in occasional Kupffer cells. The remaining three rabbits (Nos. 10, 11, and 12) died after having received numerous injections of sodium polyanetholsulfonate during periods of 24, 25, and 45 days, respectively. The postmortem findings in Rabbit 11 were much like those of Rabbit 9, there being numerous adhesions between the glomerular tufts and capsules and slight intercapillary sclerosis in addition to small intracapillary precipitates in alveolar septa and numerous macrophages, with ingested granular deposits in spleen and lung. In addition to displaying various stages of glomerular sclerosis with fibrosis and other changes like those already described, the kidneys of Rabbit 10 and those of Rabbit 12 exhibited hemorrhagic necrosis of many of the glomeruli and coagulation necrosis of many of the cortical tubules, and Rabbit 12 had, in addition, an early cirrhosis of the liver, with slight fatty metamorphosis, and a meningoencephalitis, with organisms resembling toxoplasma. The renal changes just described were interpreted as being secondary to the formation of the intravascular precipitates following injection of the sodium polyanetholsulfonate, with occlusion of the capillaries of the glomeruli giving rise to glomerular sclerosis in the less severe instances and to necrosis of glomeruli and tubules in those more severe. More extensive studies of these secondary renal lesions are now being made.

## COMMENT

In 1930, Demole and Reinert injected sodium polyanetholsulfonate experimentally in rabbits and noted widespread and extensive hemorrhages involving heart, lungs, adrenals, intestines, and skeletal musculature; they attributed death of their animals to respiratory paralysis.[4] They also reported a "glomerulonephritis with involvement of glomeruli and tubules" in some of their animals but did not provide descriptions or illustrations of the lesions. It is noteworthy that the sodium polyanetholsulfonate used by Demole and Reinert may have been somewhat different from that utilized in the present studies, since it was described as being a light brown powder; whereas the lethal dose was found to be 50 mg. per kilogram of body weight in rabbits. The findings of the present work suggest that the sodium polyanetholsulfonate brought about a precipitation of one or another of the proteins of the blood and that the coagulated material accumulated conspicuously in the capillaries of the lungs and kidneys, the vascular occlusion in the former situation bringing about death within a few hours when a relatively large dose (for example, 15 mg. per kilogram of body weight) was given; whereas those in the latter situation gave rise to curious anatomical changes resembling the "wire-loop" lesions seen in disseminated lupus erythematosus and resulting, if the animal survived, in sclerosis of the glomerular tufts and necrosis of glomeruli and of tubular epithelium as well.

4. Demole, V., and Reinert, M.: Polyanetholsulfosäures Natrium: Ein neues synthetisches Mittel zur Hemmung der Blutgerinnung, Arch. exper. Path. u. Pharmakol. **158**:211, 1930.

Intracapillary precipitates like those described in the present paper have not been encountered in the kidneys or other viscera of the many normal rabbits examined post mortem in this laboratory during the past several years, or have they been seen in the viscera of numerous rabbits with other experimental lesions (for example, neoplasms, periarteritis nodosa, experimental siderosis, and experimental hyperglobulinemia with amyloidosis). Although the occlusion of glomerular capillaries by thrombi or casts is frequently mentioned as occurring in experimental nephritis produced in a variety of ways,[5] the thrombi or casts are usually small, and they are by no means constantly seen; on the whole they have but little resemblance to the intracapillary precipitates encountered in the present work. Glomerular precipitates similar to the ones described in this paper have been produced experimentally, however, by Masson, Corcoran, and Page,[6] who injected renin subcutaneously into rabbits in which one kidney had been removed and in which hypertension had followed the injection of desoxycorticosterone acetate. The glomerular lesions they describe and illustrate are remarkably like the acute ones of the present study. Thomas and Good[7] and Schwab, Watson, and Chromartie[8] have likewise noted similar precipitates in the glomerular capillaries of rabbits in which a generalized Shwartzman reaction had been induced.

SUMMARY

Adult rabbits died abruptly within a few hours when a relatively large dose of sodium polyanetholsulfonate (Liquoid) was given intravenously to them (for example, 15 mg. per kilogram of body weight), and microscopic studies showed that intracapillary precipitates were regularly present in the lungs, kidneys, and other organs. The finding of numerous precipitates forming casts in the capillaries of the alveolar septa, together with marked pulmonary congestion and edema and widespread petechial hemorrhages and ecchymoses, and the absence of such precipitates in the blood vessels of the brain suggested that occlusion of the pulmonary capillaries had brought about death.

Intracapillary precipitates were likewise numerous in the glomeruli of the rabbits that had died abruptly after receiving sodium polyanetholsulfonate. Often they lined the walls of the capillaries and formed casts which resembled to some extent the "wire-loop" lesions seen in disseminated lupus erythematosus.

5. Ehrich, W. E.; Forman, C. W., and Seifer, J.: Diffuse Glomerular Nephritis and Lipid Nephrosis: Correlation of Clinical, Morphological, and Experimental Observations, A. M. A. Arch. Path. **54**:463, 1952. Christian, H. A.: Non-Specificity of Glomerular Lesions of the Kidney, Am. J. M. Sc. **204**:781, 1942.

6. Masson, G. M. C.; Corcoran, A. C., and Page, I. H.: Renal and Vascular Lesions Elicited by "Renin" in Rats with Desoxycorticosterone Hypertension, A. M. A. Arch. Path. **53**:217, 1952.

7. Thomas, L., and Good, R. A.: The Effect of Cortisone on the Shwartzman Reaction: The Production of Lesions Resembling the Dermal and Generalized Shwartzman Reaction by a Single Injection of Bacterial Toxin in Cortisone-Treated Rabbits, J. Exper. Med. **95**:409, 1952.

8. Schwab, J. H.; Watson, D. W., and Chromartie, W. J.: Production of Generalized Shwartzman Reaction with Group A Streptococcal Factors, Proc. Soc. Exper. Biol. & Med. **82**:754, 1953.

Rabbits that survived for some days after one or more injections of smaller doses of sodium polyanetholsulfonate regularly manifested upon postmortem examination a glomerular sclerosis of greater or less extent and sometimes necrosis of glomeruli and tubules as well, the changes being interpreted as resulting from the occlusion of renal capillaries by the precipitates.

Numerous macrophages containing ingested granular eosinophilic material in their cytoplasm were seen in the spleens and lungs of rabbits that had survived one or more injections of sodium polyanetholsulfonate, and the Kupffer cells often contained similar material. Early cirrhosis of the liver was seen in one rabbit that died after receiving numerous injections of sodium polyanetholsulfonate.

Brief reference is made to the occurrence of intracapillary precipitates under other experimental conditions.

The photomicrographs were made by Julius Mesiar.

# CALCIUM AND MAGNESIUM CONTENT OF ALBUMINOID FRACTION OF HUMAN AORTAE

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EXPERIMENTS designed to elucidate some of the underlying chemical changes associated with aging and atherosclerosis of elastic arteries by means of analyses of the albuminoid (collagen, elastin, and reticulin) fraction of the human aorta have recently been performed in this laboratory. A carbohydrate component of this protein fraction which is not quantitatively changed with progressive age or atherosclerosis has been observed.[1] The aortic albuminoid combines with equal amounts of sodium hydroxide, regardless of differences in age or atherosclerotic condition of the aorta, but combines with larger quantities of hydrochloric acid when prepared from aged and atherosclerotic aortae.[2] It was then suggested that the acid-combining properties may be correlated, at least in part, with the mineral contents of these aortae. Except for the above changes in acid-combining properties, no differences were noted in the swelling and titration curves of albuminoids from aortae of varying age and degree of atherosclerosis.[3] Analyses of the elastin content of this albuminoid fraction revealed a significant increase only in aortae observed grossly to contain calcified plaques.[4] It was then suggested that mineral depositions might be responsible for this change. It appeared necessary, therefore, to investigate the mineral contents of the albuminoid and elastin fractions of human aortae.

Determinations of the calcium and magnesium content of whole aortae have been performed by Buck,[5] who demonstrated that progressive aging and atherosclerotic processes are accompanied by increased mineral concentrations. He also presented an excellent review of the pertinent literature. Lansing and co-workers have shown a progressive increase with aging in the calcium content of elastin prepared from the aortic media of persons between the ages of 20 and 50.[6]

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1. Miller, H.; Haft, H., and Kraemer, D. M.: Proc. Soc. Exper. Biol. & Med. **79**:411 1952.
2. Miller, H., and Kraemer, D. M.: Proc. Soc. Exper. Biol. & Med. **80**:243, 1952.
3. Miller, H., and Kraemer, D. M.: Proc. Soc. Exper. Biol. & Med. **83**:209, 1953.
4. Kraemer, D. M., and Miller, H.: A. M. A. Arch. Path. **55**:70, 1953.
5. Buck, R. C.: A. M. A. Arch. Path. **51**:319, 1951.
6. Lansing, A. I.; Alex, M., and Rosenthal, T. B.: J. Gerontol. **5**:112, 1950.

## MATERIAL AND METHODS

The albuminoid fractions of thoracic aortae obtained at autopsy were prepared by previously described methods.[4] Between 0.5 and 3.2 gm. of albuminoid from each aorta was weighed on an analytical balance and analyzed for calcium and magnesium. From 0.4 to 1.4 gm. samples of elastin were prepared by methods previously described [4] from different aliquots of the same aortae. The elastin components were weighed and analyzed for calcium and magnesium.

All samples were ashed at 250 to 300 C. in silica crucibles for several hours or until they were well carbonized. The temperature was then raised to 500 to 600 C. until the ash was white or slightly gray in color. After cooling, the ash was dissolved in 1 N hydrochloric acid, using near boiling temperatures when necessary to effect dissolution. The volume was then made up to 25 ml. with distilled water in a volumetric flask. This stock solution was further diluted so that 1 ml. contained 30 to 600 $\gamma$ of calcium and 2 to 34 $\gamma$ of magnesium. Calcium was estimated by the method of Sobel and Sobel [7] and magnesium by the method of Hirschman.[8] The latter method is a spectrophotometric determination of excess 8-hydroxyquinoline following precipitation of magnesium with a known quantity of 8-hydroxyquinoline. The trivalent metals and calcium were removed previously.

## RESULTS

The analytical data were classified according to age and degree of atherosclerosis (Tables 1 and 2). The 56 to 71 year age groups and the pathological group characterized by the presence of a few calcified plaques, as well as atheromatous plaques, were used as base lines for statistical analyses ($t$ tests). Scatter graphs of age as compared to mineral changes suggested an exponential relationship. Therefore, the coefficients of correlation ($r$) were calculated from the logarithms of the mineral values and the age distribution. Only probability ($P$) values less than 0.05 were considered significant.

In general, there is a progressive increase in the calcium content of both the albuminoid and the elastin components of this albuminoid with increasing age and degree of atherosclerosis (Table 1). There is a significant correlation between the log of the albuminoid calcium and age ($r = 0.66$, $P = < 0.01$). Increasing degrees of atherosclerosis are accompanied by increases in albuminoid calcium. The mean values obtained from the "normal" and atheromatous groups are significantly less than those from the aortae showing few calcified plaques. Aortae containing many calcified plaques contain significantly more albuminoid calcium than those with only few calcified plaques. The log of the calcium content of the elastin fraction of the albuminoid increases significantly with increasing age ($r = 0.88$, $P = < 0.001$). The greater concentrations of calcium in the elastin of aortae showing macroscopic calcifications are significantly different from the "normal" group.

Magnesium, with two apparent exceptions, also shows a trend towards higher concentrations in the albuminoid fraction and elastin component of this albuminoid with progressive age and atherosclerosis (Table 2). The log of the albuminoid magnesium content undergoes a significant increase with increasing age ($r = 0.69$, $P = < 0.001$). The albuminoid magnesium content of the group containing only atheromatous plaques is significantly less than that of the group also containing few visible calcifications. The trend towards decreasing magnesium concentration in the highly atherosclerotic group is of interest, since the same samples showed a highly significant, almost twofold increase in calcium when compared with the group containing only few calcified plaques. The log of the magnesium content of

7. Sobel, A. E., and Sobel, B. A.: J. Biol. Chem. **129**:721, 1939.

8. Hirschman, A.: Unpublished data.

the elastin fraction increased significantly with increasing age ($r = 0.76$, $P = < 0.001$). The "normal" group differs significantly from the pathological group containing few calcified plaques in respect to elastin magnesium.

Since the albuminoid of the aorta may be considered, on the basis of sodium hydroxide hydrolysis, to be composed of collagen and elastin fractions, it is possible

TABLE 1.—*Calcium Content of Albuminoid Fraction of Twenty-One Human Aortae Grouped According to Age and Atherosclerotic Condition*

| | Albuminoid | | | | | Elastin | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Age, Yr. | No. of Samples | Mean Ca Content, Mg./Gm. | Standard Deviation | $t$ | Probability | No. of Samples | Mean Ca Content, Mg./Gm. | Standard Deviation | $t$ | Probability |
| 24-39 | 2 | 1.5 | 0.16 | 1.68 | $>0.1$ | 2 | 4.4 | 1.4 | 1.73 | $>0.1$ |
| 40-55 | 3 | 19.7 | 19.4 | 0.67 | $>0.1$ | 3 | 42.7 | 30.5 | 1.11 | $>0.1$ |
| 56-71* | 8 | 19.0 | 14.1 | .... | ..... | 8 | 67.3 | 49.2 | .... | ..... |
| 72-86 | 8 | 24.8 | 13.5 | 0.84 | $>0.1$ | 7 | 84.4 | 42.8 | 0.72 | $>0.1$ |
| Atherosclerosis† | | | | | | | | | | |
| "Normal" | 2 | 1.5 | 0.16 | 3.62 | $<0.01$ | 2 | 4.4 | 1.4 | 4.45 | $<0.01$ |
| Atheromatous plaques | 5 | 7.4 | 5.0 | 3.44 | $<0.01$ | 5 | 49.4 | 66.4 | 1.08 | $>0.1$ |
| Few calcified plaques and atheromatous plaques* | 8 | 19.4 | 6.7 | .... | ..... | 7 | 73.9 | 31.4 | .... | ..... |
| Many calcified plaques and atheromatous plaques | 6 | 36.4 | 9.6 | 3.98 | $<0.01$ | 6 | 82.1 | 36.1 | 0.51 | $>0.1$ |

* Group used as the base line for calculation of the $t$ values.
† Classification based upon gross observations.

TABLE 2.—*Magnesium Content of Albumoid Fraction of Twenty-One Human Aortae Grouped According to Age and Atherosclerotic Condition*

| | Albuminoid | | | | | Elastin | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Age, Yr. | No. of Samples | Mean Mg. Content, Mg./Gm. | Standard Deviation | $t$ | Probability | No. of Samples | Mean Mg. Content, Mg./Gm. | Standard Deviation | $t$ | Probability |
| 24-39 | 2 | 0.16 | 0.04 | 1.28 | $>0.1$ | 2 | 0.35 | 0.32 | 2.64 | $<0.05$ |
| 40-55 | 3 | 0.74 | 0.30 | 0.92 | $>0.1$ | 3 | 3.95 | 4.05 | 1.45 | $>0.1$ |
| 56-71* | 8 | 1.55 | 1.46 | .... | ..... | 8 | 2.45 | 1.07 | .... | ..... |
| 72-86 | 8 | 1.88 | 1.69 | 0.42 | $>0.1$ | 7 | 2.98 | 0.97 | 0.99 | $>0.1$ |
| Atherosclerosis† | | | | | | | | | | |
| "Normal" | 2 | 0.16 | 0.04 | 1.61 | $>0.1$ | 2 | 0.35 | 0.32 | 7.58 | $<0.01$ |
| Atheromatous plaques | 5 | 0.53 | 0.35 | 2.11 | $<0.1$ | 5 | 1.96 | 1.63 | 1.82 | $<0.1$ |
| Few calcified plaques and atheromatous plaques* | 8 | 2.11 | 1.63 | .... | ..... | 7 | 3.00 | 0.69 | .... | ..... |
| Many calcified plaques and atheromatous plaques | 6 | 1.67 | 1.50 | 0.52 | $>0.1$ | 6 | 3.59 | 2.48 | 0.70 | $>0.1$ |

* Group used as the base line for calculation of the $t$ values.
† Classification based upon gross observations.

to calculate the mineral content of the collagen when given the percentage elastin in the albuminoid and the albuminoid and elastin mineral concentration. The percentage elastin, estimated by previously described methods,[4] for all 21 samples averaged 32. These methods, though the best available, are still somewhat crude. The mean collagen calcium content was calculated to be—0.74 mEq. per gram, and the mean collagen magnesium was 0.77 mEq. per gram. It seemed reasonable to assume that these figures were the cumulative result of minor errors in the factors involved in their calculations and that the true values should be zero. This assumption was tested by heating a pooled albuminoid sample representing 13

powdered (Wiley mill, 40 mesh) aortae with 0.1 N sodium hydroxide in a boiling water bath for one hour. The collagen hydrolysate, following ashing in concentrated nitric acid, yielded negative tests for both calcium and magnesium. Substituting values for calcium and magnesium that are 5% greater or less than the observed values does not appreciably effect the calculated mineral content of the collagen fraction. Taking this factor into consideration, as well as the relatively negligible errors resulting from weighing the albuminoid and elastin samples in determining the percentage elastin,[4] it appears likely that the greatest part of the error in calculating the mineral content of collagen is due to errors in sampling. This error, however, would not seem to be of sufficient magnitude to affect the original interpretation of the results.

Since the mineral content of the aortic albuminoid is present only in the elastin fraction when elastin is prepared by hydrolysis in dilute base, it is important to know whether or not this mineral exists in a free or protein-bound state. Since the specific gravity of non-protein-bound mineral should be appreciably greater than that of the protein fraction, it was decided to separate the two fractions by means of a liquid of relatively high specific gravity. Lansing and co-workers [9] have already found that a sucrose solution of specific gravity 1.30 will separate ground elastin obtained from the tunica media of human aortae into two fractions by differential centrifugation. The heavier fraction contained 6.39% calcium; whereas the lighter fraction was composed of only 1.14% calcium. Since the specific gravity of tricalcium phosphate (presumably the major ash component [9]) is 3.14, it was decided to centrifuge in carbon tetrachloride, which has a specific gravity of 1.60, in order to effect a more complete separation. The pooled aortic albuminoid sample referred to above was hydrolyzed for three hours in three solutions of 0.1 N sodium hydroxide in a boiling water bath, filtered under suction, washed three times with distilled water and twice each with absolute ethanol and ether, and repowdered (60 mesh) in a Wiley mill. The resulting white powder, which gave a negative anthrone test for carbohydrate,[10] was centrifuged in carbon tetrachloride at about 400g for one hour. Approximately 20% by weight of the elastin comprised the heavy layer, the remainder floating on top. The two fractions were ashed at about 250 C. for 10 hours and at about 550 C. for three hours. The ash content of the heavier layer was 86%, and that of the lighter fraction was 14%. Sixty-two per cent of the total ash is therefore in the heavy layer, which constitutes only 20% of the total weight of elastin. It seems likely that if an extremely fine powder (for example, 200 mesh) were prepared, the separation would be even greater. It therefore seems reasonable to assume that the greatest portion of the mineral content of the aortic elastin is not bound to protein.

## COMMENT

The increased acid-combining properties of the human aortic albuminoid previously observed with increasing age and degree of atherosclerosis [2] follow a trend similar to that noted for albuminoid calcium in the present investigation. The data obtained from the investigations expressed in terms of milliequivalents per gram of

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10. Seifter, S.; Dayton, S.; Novic, B., and Muntwyler, E.: Arch. Biochem. **25**:191, 1950.

albuminoid are presented in Table 3. If the hydrochloric acid combined by the albuminoid is in reality combined by calcium compounds, a constant numerical relationship between the calcium content and bound hydrochloric acid would be expected. Such a correlation is not present when the raw data are examined. However, it would seem likely that the small amount of calcium present in apparently normal aortae is protein-bound or at least not present in the form of a mineral deposition. Likewise, the hydrochloric acid combined by "normal" aortae is probably bound by protein since the albuminoid has an isoelectric range at about neutrality,[3] and the number of milliequivalents of sodium hydroxide bound by the albuminoid in all pathological groups is essentially equal to the milliequivalents of bound hydrochloric acid in the "normal" group.[2] Thus, in order to test the

TABLE 3.—*Relationship Between Calcium Content and Hydrochloric Acid-Combining Properties of Human Aortic Albuminoid*

| Degree of Atherosclerosis | Mean Ca Content, mEq./Gm. | Mean HCl Combined, mEq./Gm. | Net Ca Content,* mEq./Gm. | Net HCl Combined,* mEq./Gm. | Net mEq. Ca Net mEq. HCl | Net HCl Combined Corrected for $H_3PO_4$, mEq./Gm. |
|---|---|---|---|---|---|---|
| "Normal" | 0.08 | 0.77 | .... | .... | .... | .... |
| Atheromatous plaques.. | 0.37 | 0.95 | 0.29 | 0.18 | 1.61 | 0.30 |
| Few calcified plaques.... | 0.97 | 1.3 | 0.89 | 0.53 | 1.68 | 0.91 |
| Many calcified plaques.. | 1.8 | 1.8 | 1.7 | 1.0 | 1.7 | 1.7 |

* Obtained by subtracting the "normal" value from the mean value.

TABLE 4.—*Relation Between Calcium Content and Percentage Elastin of Human Aortic Albuminoid*

| Degree of Atherosclerosis | Mean Ca Content, Mg. $Ca_3(PO_4)_2$/ Gm. Albuminoid | Mean Elastin Content, Mg./ Gm. Albuminoid | Mean Elastin Corrected for $Ca_3(PO_4)_2$, Mg./ Gm. Albuminoid |
|---|---|---|---|
| "Normal" | 3.9 | 308 | 304 |
| Atheromatous plaques | 19.1 | 325 | 306 |
| Few calcified plaques | 50.1 | 365 | 315 |
| Many calcified plaques | 93.9 | 417 | 323 |

hypothesis that the acid bound by atherosclerotic aortae is bound by the calcified deposit, the "normal" calcium and hydrochloric acid values have been subtracted from the atherosclerotic data to give net calcium and hydrochloric acid values (Table 3). A relatively constant relationship now exists between the calcium content and bound hydrochloric acid. However, a 1 : 1 ratio would be expected if calcium were to account for all the hydrochloric acid bound. This can be explained by making a second hypothesis based on some experimental evidence [9] that the calcium is present in the form of tricalcium phosphate or an apatite with the same empirical formula. Thus, the combined hydrochloric acid data can be corrected for hydrogen ions donated by the phosphoric acid formed in the reaction between the tricalcium phosphate and hydrochloric acid. The number of milliequivalents of phosphoric acid hydrogen titrated by sodium hydroxide at the end-point of methyl red [2] was calculated and added to the net combined hydrochloric acid figures. These values were essentially in agreement with the net calcium content of each atherosclerotic group (Table 3). Though direct proof has not been offered at the

present time, the data obtained are consistent with the hypothesis that the added calcium in atherosclerotic aortae is present mainly in the form of tricalcium phosphate.

It was stated that the statistically significant increase in mean percentage of elastin in the albuminoid fraction of atherosclerotic aortae observed grossly to contain calcium is possibly due to this increased mineral content.[4] If the calcium is present in the form of an apatite or tricalcium phosphate, this assumption seems to be correct (Table 4). More than 80% of the increase in elastin with increasing degrees of atherosclerosis can be accounted for by correcting for calcium in this form. Water of hydration, which is difficult to eliminate in this type of compound, and other minor constituents may account for much of the remainder.

The magnitude of values for the calcium content of elastin in the present investigation is essentially in agreement with those previously obtained for elastin in the tunica media of human aortae.[6] In comparing these data, it is interesting to note that, while the calcium content of the tunica media tends to level off at about 6% after the fifth decade, it continues to increase in the whole aorta, reaching a peak of over 8% in the seventh and eighth decades (Table 1). This discrepancy is probably due to increased intimal calcification in the older age groups.

## SUMMARY

A progressive increase, in general, in the contents of calcium and magnesium in both the human aortic albuminoid and the elastin fractions of this albuminoid with increasing age and degree of atherosclerosis has been demonstrated.

With progressing age and atherosclerosis, almost all of this mineral is probably deposited in an inorganic form, only a very small proportion being organically bound.

This mineral probably accounts for previously observed increases in the acid-combining properties and in the apparent elastin content of the aortic albuminoid accompanying progressive aging and atherosclerosis.

Technical assistance was given by Miss Grace O'Brien and Miss Jean Wright.

# EXPERIMENTAL THYROID TUMORIGENESIS IN RATS

## Predominance of Neoplasm Type and Influence of Age

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THE PRESENT report is part of a study that has been in progress for several years. Since the literature contains little or no data concerned with age as a factor influencing induced mammalian thyroid tumorigenesis, our experiments were designed to determine the tumor-producing effect of propylthiouracil during various age periods of the rat. We wished to acquire information regarding the incidence of thyroid neoplasia in these age groups, as well as the kind of tumor most readily induced and, if possible, the manner of its development.

Some of the neoplasms produced in this way probably have been derived from the ultimobranchial tissue component of the thyroid gland. This concept of thyroid tumorigenesis, therefore, is worthy of consideration. It is especially important to the clinician, perhaps, in view of his experiences with prolonged stimulation of the human thyroid gland during certain age periods.

Spontaneous thyroid neoplasia in the rat has been regarded generally as rare (Bullock and Curtis [1]). These investigators examined many thousands of rats, yet found only two animals that had developed thyroid tumors. One was a benign adenoma; the other, a malignant growth, was found in a rat approximately 31 months old. In 1940, Ratcliffe [2] reported on tumors in two colonies of rats at the Wistar Institute, but he mentioned no spontaneous-occurring thyroid tumors. Such thyroid neoplasia, however, has been reported in relatively small numbers of old rats (Slye, Holmes, and Wells [3]; Wegelin,[4] and Van Dyke [5]). In 1944, the writer described a large percentage of spontaneous thyroid tumors (cystadenomata), which probably were derived from cystic manifestations of ultimobranchial tissue,

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2. Ratcliffe, H. L.: Spontaneous Tumors in 2 Colonies of Rats of the Wistar Institute of Anatomy and Biology, Am. J. Path. **16**:237-254, 1940.

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in very old albino rats of a local strain. These animals were approximately 30 months of age or older. Recently the writer has also observed a substantial number of thyroid neoplasms of a particular kind in female Wistar rats 22 to 25 months of age. These were unusual new growths that possessed an anaplastic, solid, or epithelioid character.

With the advent of special experimental diets and particularly following the use of thiourea and other goitrogenic drugs, evidence has been accumulating rapidly which demonstrates some of the pathological changes involved in the production of thyroid neoplasia.

Wegelin [4] was among the first to report the occurrence of thyroid adenomata in rats following experimental use of diets which could induce thyroid hyperplasia. In 1935, Hellwig [6] produced thyroid tumors in rats by feeding an iodine-deficient but calcium-rich diet for a period of only four months. Griesbach, Kennedy, and Purves [7] showed that diets rich in brassica seed not only were goitrogenic but also could induce multiple thyroid adenomata. Continuous feeding of this diet caused an iodine deficiency and resulted in prolonged hyperplasia of the thyroid parenchyma. In 1947, Purves and Griesbach [8] induced a high percentage of thyroid neoplasms after administration of thiourea alone for periods of one year or more. After 20 months such tumors showed evidence of malignancy. Other reports of thyroid adenomata, using a goitrogenic drug alone, have appeared in the literature (Money,[9] Money and Rawson [10]).

Some investigators have produced both benign and malignant tumors in the thyroid gland of rats by combining the action of a goitrogen with that of a carcinogenic agent (Bielschowsky [11]; Paschkis, Cantarow, and Stasney [12]; Hall [13]). In 1950, Money and Rawson [14] induced thyroid adenomata by prolonged thiouracil administration accompanied by injections of dibenzanthracene. They attempted to produce definite lesions and thus determine the morphological stages through which thyroid tissue would progress in developing abnormalities which lead toward neoplasia. However, they were unable to prove any definite way in which thyroid tumors developed, but they agreed with Wegelin that thyroid tumors were invariably

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11. Bielschowsky, F.: Tumours of the Thyroid Produced by 2-Acetyl-Amino-Fluorene and Allyl-Thiourea, Brit. J. Exper. Path. **25**:90-95, 1944; Experimental Nodular Goitre, ibid. **26**:270-275, 1945.

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13. Hall, W. H.: The Role of Initiating and Promoting Factors in the Pathogenesis of Tumours of the Thyroid, Brit. J. Cancer **2**:273-280, 1948.

14. Money, W. L., and Rawson, R. W.: The Experimental Production of Thyroid Tumors in the Rat Exposed to Prolonged Treatment with Thiouracil, Cancer **3**:321-335, 1950.

preceded by parenchymatous hyperplasia. It is important to note that this experimental thyroid tumorigenesis, utilizing these various procedures, has resulted in demonstrable neoplasms of more than one kind.

In this paper attention is called to two kinds of epithelial thyroid neoplasia. One of these is a solid anaplastic type of neoplasm which occurs spontaneously in certain old Wister rats. Experimentally, large numbers of these growths can be produced in rats maintained on a goitrogen combined with a carcinogenic agent during chronic vitamin A deficiency. Preliminary accounts of these neoplasms have been reported.[15] A complete description of their development from the ultimobranchial tissue component of the thyroid gland will be presented shortly in another communication.

A second type of tumor, the more common spontaneous thyroid neoplasm in senile rats, is essentially that of the hyperchromatic adenoma. When induced experimentally, their incidence appears to be influenced by the age of the animal. Such tumors, many of which were papillary and cystic (cystadenomata), were the predominanting neoplasms following continuous administration of propylthiouracil. To facilitate a recognition of the initial position of these tumors and their relationship to ultimobranchial tissue within the thyroid gland, particular attention has been paid to the induction of incipient neoplasms.

## MATERIAL AND METHODS

In order to determine the extent to which age influences the development of experimental thyroid tumors, only rats originating from the Wistar stock strain (females) and bred in our laboratory were employed. Wistar rats were used because spontaneous thyroid tumorigenesis is apparently rare in these animals.[2]

A total of 84 animals were utilized in the present study. Thirty-six of these were set aside as normal controls. These were killed in separate groups at 4, 8, and 20 months of age (Table 1). The remaining 48 rats were maintained continuously on 0.2% propylthiouracil in stock diet for different periods of time. The ages of these animals varied from 2 to 15 months at the beginning of the experiment. They were fed the goitrogenic diet for periods ranging from 2 to 14 months. However, their average age at the start of the experiment and the average time these rats were subjected to propylthiouracil are indicated in four groups in Table 2. The average age of the oldest experimental animals was 16 months at the end of the experiment (i. e., some four months younger than the oldest control rats).

As recommended by Paletta and Cowdry[16] for the mouse, all animals were subjected to 0.01% aqueous solution of colchicine seven hours before autopsy to demonstrate evidence of mitoses under optimum conditions. The entire larynx and thyroid gland of each animal were fixed in either Bouin's fluid, Zenker's fluid, or 10% neutral formalin. Serial paraffin preparations were stained by either the hematoxylin and eosin, Mallory's connective tissue, or silver impregnation technique.

## OBSERVATIONS AND RESULTS

*Thyroid Glands in Normal (Control) Rats.*—Observations on the thyroid gland of control Wistar rats, at least at the ages examined, substantiate the concept that spontaneous thyroid neoplasia is uncommon in these animals. In two instances only were thyroid neoplasms identified (5.5% of the total). These were small single new growths which occurred in the oldest rats (Table 1) and might well have been overlooked if serial sections had not been employed in this study. Moreover, these

15. Van Dyke, J. H.: Experimental Thyroid Tumors Derived from Ultimobranchial Tissue in the Rat, Proc. Indiana Acad. Sc. (1950) **60**:313-314, 1951; Influence of Age on Experimental Thyroid Tumorigenesis in Female Rats, abstracted, Anat. Rec. **115**:377, 1953.

16. Paletta, F. X., and Cowdry, E. V.: Influence of Colchicine During Methylcholanthrene Epidermal Carcinogenesis in Mice, Am. J. Path. **18**:291-311, 1942.

neoplasms represented two distinctly different types, and they were similar to certain tumors produced experimentally by others (Money and Rawson,[14] Bielschowsky [11]).

One of the two spontaneous thyroid neoplasms was questionable. Nevertheless, it resembled that type illustrated in Figure 4 and has been regarded as an incipient cystadenoma. The other spontaneous neoplasm was an anaplastic, solid, or epithelioid type of growth similar to that pictured in Figure 6. Mitoses were not conspicuous in either of these tumors. They were surrounded by a delicate connective tissue capsule.

In a previous study the writer found that spontaneous thyroid cystadenomata in senile rats were frequently associated with cysts lined by stratified squamous epithelium. The evidence suggested that certain cells in the walls of these cysts (probably suprabasal cells) gave origin to some of these thyroid tumors. Because of the position of these epithelial cysts in or near the center of each lobe of the thyroid gland and their relationship to the parathyroid glands, they have been interpreted as arising, through metaplasia, from the ultimobranchial tissue component (Van Dyke [17]). Similar metaplastic manifestations have also been described in apparently normal thyroids of sheep (Van Dyke [18]).

TABLE 1.—*Number and Character of Spontaneous Thyroid Neoplasms in Thirty-Six Normal (Control) Female Wistar Rats of Various Ages*

| No. of Rats | Average Age, Mo. | Adenomata | |
|---|---|---|---|
| | | Solid | Cystic |
| 10 | 4 (young) | 0 | 0 |
| 15 | 8 (adult) | 0 | 0 |
| 11 | 20 (old) | 1 | 1* |
| Totals 36 | | 1 | 1 |

* Questionable neoplasm.

In rats, as in sheep, ultimobranchial cysts can give rise to large gland-like cells which come to lie initially between the cyst wall and its connective tissue capsule. As cell aggregates, they resemble young thyroid tissue. In hyperplastic thyroid glands such new growths form more or less typical thyroid vesicles which contribute to the general thyroid parenchyma.

On the other hand, in atrophic thyroids these cell clusters usually form solid epithelioid masses, very much like the experimental adenoma in Figure 6. This cell type originates singly or in clumps from the germinative cell layers of stratified squamous epithelium lining the walls of these cysts. The "stripping" of these basal cells, however, results in thin-walled cysts made up almost wholly of suprabasal cell layers. As such, these cysts may persist indefinitely. As previously pointed out,[5] however, the very existence of these ultimobranchial cysts is usually incompatible with experimentally induced thyroid stimulation (i. e., they tend to disappear during prolonged periods of hyperplasia).

17. Van Dyke, J. H.: The Development and Activity of Ultimobranchial Cysts in the Thyroid Gland of Rats on a Vitamin A Deficient Diet, abstracted, Anat. Rec. **82**:451, 1942; footnote 5.

18. Van Dyke, J. H.: Behavior of Ultimobranchial Tissue in the Postnatal Thyroid Gland: Epithelial Cysts; Thier Relation to Thyroid Parenchyma and to "New-Growths" in the Thyroid Gland of Young Sheep, Am. J. Anat. **76**:201-251, 1945.

Similar cysts were noted in a number of thyroid glands of the control animals. Nevertheless, few such instances of thyroid metaplasia occurred in histologically normal glands. Ultimobranchial cysts were observed mostly in the young and old rats, i. e., in thyroids where the parenchyma appeared to have been either hyperplastic or atrophic. In general, cysts in young animals were thick-walled and showed evidence of mitosis in their basal cells. They appeared to contribute new cells to the thyroid gland. In older rats such cysts were thin-walled and represented, largely, persisting suprabasal cells. However, the two spontaneous thyroid neoplasms in the oldest control animals were not associated with ultimobranchial cysts.

*Thyroid Glands in Rats Given Propylthiouracil.*—Examination of thyroid glands in rats given 0.2% propylthiouracil over extended periods indicates that such stimulation produces extreme histological changes. The result of feeding such a tumorigenic diet is indicated in Table 2. This Table shows the average age of four groups of female rats before treatment with the goitrogen and the average period of time each age group was maintained on the experimental diet. Two different kinds of thyroid neoplasia were encountered, as indicated in Table 2.

TABLE 2.—*Number and Character of Experimental Thyroids Exhibiting Neoplasms in Forty-Eight Female Wistar Rats**

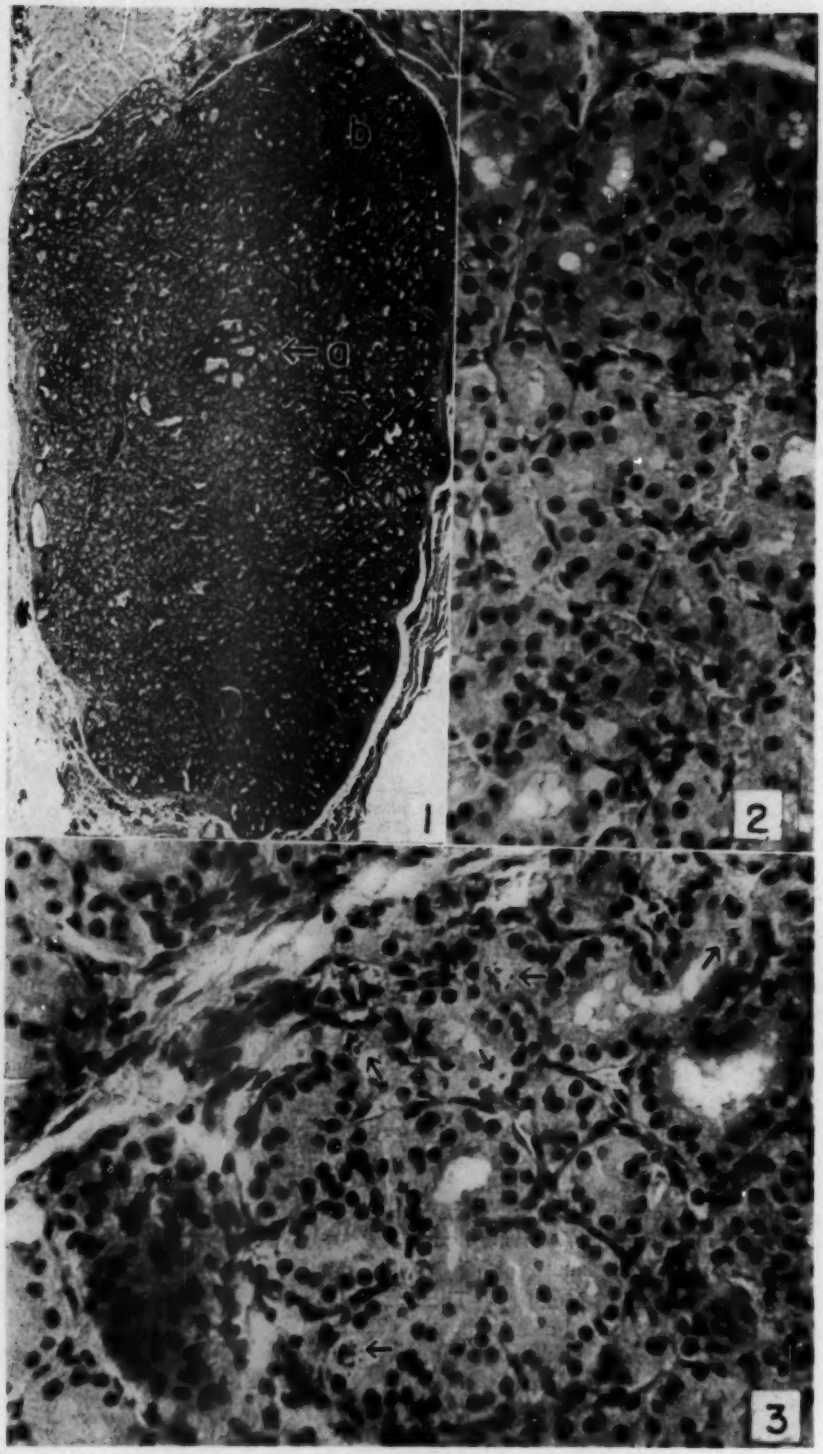
| Group | No. of Animals | Average Age, Mo. | Average Experimental Time, Mo. | Average Total Age, Mo. | Adenomata | |
|---|---|---|---|---|---|---|
| | | | | | Solid | Cystic |
| 1 | 15 | 2.5 | 3 | 5.5 | 0 | 1† (7%) |
| 2 | 12 | 10.2 | 4 | 14.2 | 1 | 8 (66%) |
| 3 | 12 | 2.0 | 13 | 15.0 | 2‡ | 7§ (58%) |
| 4 | 9 | 5.0 | 11 | 16.0 | 1 | 4§ (44%) |
| Totals | 48 | ... | .. | ... | 4 (8%) | 20 (42%) |

* Groups of different age were maintained on 0.2% propylthiouracil in stock diet for variable periods.
† Questionable neoplasm.
‡ Single bilateral neoplasm in one rat.
§ Single bilateral neoplasms in three rats.

GROUP 1 (Age 2.5 Months).—These animals were fed the goitrogenic diet for an average period of three months (Table 2). The thyroid parenchyma here generally showed moderate intrafollicular growth characterized by the formation of papillary processes. The cell height of follicles was increased, but the lumen of these vesicles was usually not occluded. Colloid, while markedly reduced, was not totally absent, for remnants could be found in many follicles.

Only one animal showed evidence of thyroid neoplasia (7% of the total). This was again a questionable neoplasm composed of only a few hyperchromatic vesicles. Typical ultimobranchial cysts were identified in one or two lobes of the thyroid in four of these young rats. As in the case of apparently normal but hyperplastic thyroids, these cysts appeared to have contributed new cells to the adjacent thyroid parenchyma. However, there was no evidence to suggest that they were involved in any neoplastic change. Mitotic figures were uniformly conspicuous throughout most of these thyroid glands.

GROUP 2 (Age 10.2 Months).—These animals were kept on the propylthiouracil diet for an average period of four months (Table 2). The thyroid parenchyma was similar in character to that seen in animals of Group 1, although mitotic figures were less uniformly distributed in the glands. Nine single adenomata were encoun-




Figures 1-3

*(See legends on opposite page)*

tered. One of these was of the solid or epithelioid type resembling that shown in Figure 6. Eight tumors (66%) were in the form of hyperchromatic cystadenomata. Two neoplasms were incipient, the earliest occurring after two months on the special diet (Table 3).

Figure 1 illustrates a locus of incipient aberrant follicles in the center of one lateral lobe of the thyroid gland (*a*). It will be noted that these hyperchromatic aberrant follicles are also in the plane of section of the parathyroid gland (*b*). Using the criteria previously stated, the position of this new growth strongly suggests an origin from the ultimobranchial tissue component of the thyroid gland. Furthermore, a comparison of Figures 2 and 3, representing a high-power field of the general thyroid parenchyma (Fig. 2) and the site of the young adenoma (Fig. 3), respectively, reveals that a maximum number of mitoses occurred in the center of this thyroid lobe (*arrows*). This is the position in which ultimobranchial tissue normally would be expected to lie.

A typical early cystadenoma with papillary formations is shown in Figure 9. Since the predominating tumor in this study is essentially of this type, the following description will suffice for all instances of similar experimental neoplasia. Histo-

TABLE 3.—*Number of Rats with Incipient and Multiple Cystadenomata (Within One Lobe) in Four Experimental Groups*

| Group | Incipient Neoplasms | Earliest Neoplasm | Multiple Neoplasms |
|---|---|---|---|
| 1 | 1* | 4 mo. | 0 |
| 2 | 2 | 2 mo. | 0 |
| 3 | 5 | 9 mo. | 3 |
| 4 | 1 | 7.5 mo. | 2 |

* Questionable neoplasm.

logically they possessed cells that were hyperchromatic and usually lower than normal in cell height. Many of the cells contained densely staining pyknotic nuclei, and some appeared to be dividing by a process of amitosis. Young tumors consisted of relatively small vesicles of irregular size and shape. They showed little or no evidence of papillary formations (Figs. 1, 4, and 11). The larger, more mature cystadenoma often showed extensive papillary processes and usually formed multiple cysts lined by a low columnar epithelium (Figs. 5 and 7). Colloid in these tumors was usually brittle and poorly stained with acid dyes. The colloid occasionally took a basic stain. Such tumors were highly vascularized (Figs. 8, 11, and 13*bv*).

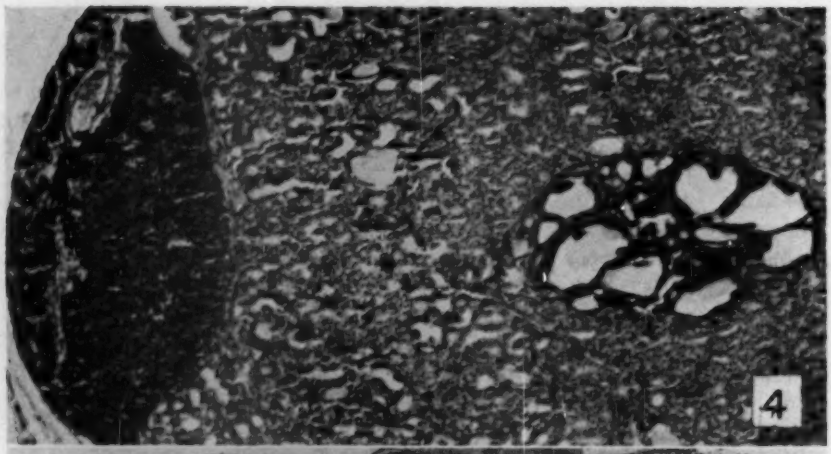
Another neoplasm in this group is demonstrated in Figure 10. Here the tumor occurred at the periphery of the thyroid (*a*). It was somewhat removed both from

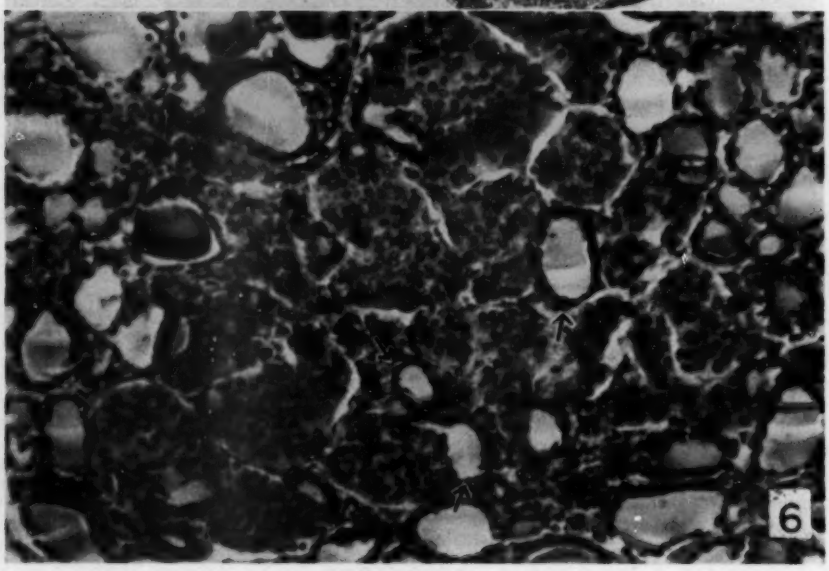
EXPLANATION OF FIGURES 1-3

Fig. 1.—Locus of aberrant follicles (*a*) within a thyroid lobe (incipient adenoma). Note that these follicles are centrally located and at level of parathyroid gland (*b*). Female rat (7 months old) maintained on propylthiouracil for five months. Hematoxylin and eosin; × 45.

Fig. 2.—High magnification of a field somewhat removed from centrally located aberrant follicles illustrated in Figure 1. Note activation of general thyroid parenchyma but the absence of mitotic figures. Hematoxylin and eosin; × 265.

Fig. 3.—High magnification of a field contiguous with the aberrant thyroid follicles shown in Figure 1. Note numerous mitotic figures illustrated in this central region (*arrows*) and compare with Figure 2. Hematoxylin and eosin; × 265.






Figures 4-6

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the parathyroid gland (*b*) and from the center of the lateral lobe. However, this was not the usual location for the earliest neoplasms identified in this study. Figure 11 also illustrates the rapid growth of such neoplastic cells under high magnification. Here the cells have produced cords or "chain-like" arrangements of small alveoli (*arrows*). Of the nine neoplasms found in animals of Group 2, all of which were young growths, seven occupied a position in which the ultimobranchial body might be expected to lie.

Small ultimobranchial cysts were found in the thyroid of five rats in Group 2. Most of these were thin-walled cysts composed largely of suprabasal cells, but they frequently showed signs of hyperplasia. Cystic remnants in three thyroids were located close to cystadenomata. It was not clear, however, whether such cysts had given rise to these neoplasms or not.

GROUP 3 (Age 2 Months).—Rats of this group, which were maintained on the goitrogen for an average of 13 months, exhibited marked changes in the character of the thyroid parenchyma. Few follicles contained colloid. When present, it stained more with basic than with acid dyes. In general, the parenchyma was in the form of cords containing large swollen cells. Blood capillaries between these cords were greatly enlarged. Mitotic figures, while identified in considerable numbers, were not as numerous as in either of the foregoing groups. Of the nine animals possessing thyroid neoplasms in this group, seven were cystadenomata (58%). Three of these were single bilateral cystadenomata (Table 2). Solid (epithelioid) undifferentiated neoplasms occurred in two rats (bilateral in one). Multiple neoplasms within one lobe of the thyroid gland were found in three animals. In five rats, the thyroid demonstrated early tumorigenesis, the earliest tumor occurring after treatment for nine months (Table 3).

An early cystadenoma in this group is shown lying near the parathyroid gland in Figure 4. It also occupied a central position within the thyroid lobe. In Figure 5, a generalized cystadenomatous change is illustrated under low power in the two lobes of the thyroid gland in a rat of similar age and experimental time. This photomicrograph indicates that it is the center of each lateral lobe of the thyroid gland that is involved in this neoplastic change. This position again suggests an origin from the ultimobranchial tissue component of the thyroid gland (*arrows*). Figure 7 exhibits another cystadenoma, under high magnification, similar to that in Figure 4. However, the presence of numerous papillary processes suggests that it is a more rapidly growing tumor. Although not illustrated, this growth also occupied a central position in the thyroid and lay near the parathyroid gland.

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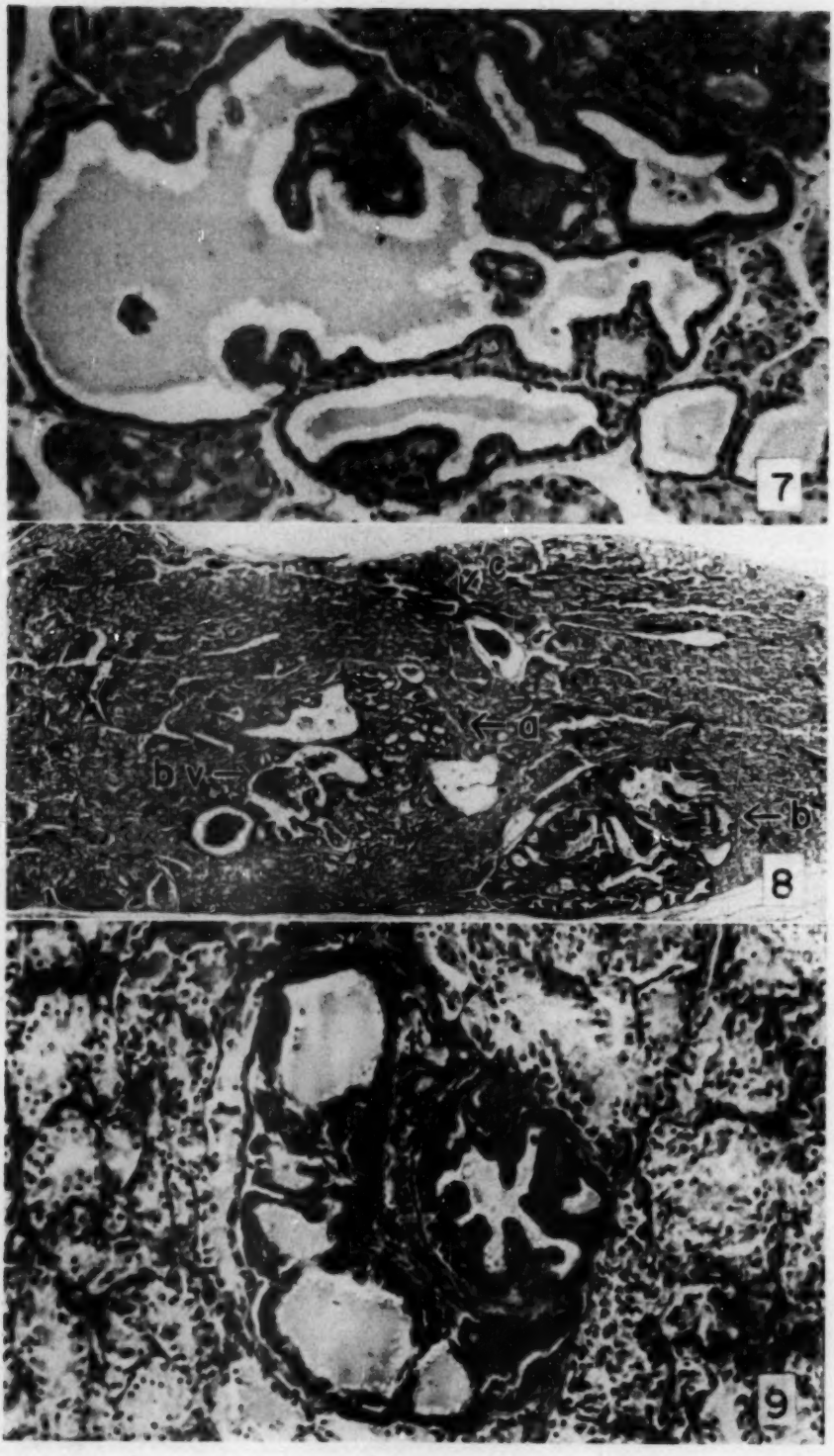
EXPLANATION OF FIGURES 4-6

Fig. 4.—Small (early) cystadenoma in center of thyroid lobe. Note its relationship to the parathyroid gland. A cystadenoma was also found in the opposite lobe. Female rat (1 month old) given propylthiouracil for 14 months. Hematoxylin and eosin; × 60.

Fig. 5.—Low-power section through center of the two lobes of the thyroid gland. Note position of developing adenomata in the center of each lateral lobe (*arrows*). Female rat (1 month old) given propylthiouracil for 14 months. Hematoxylin and eosin; × 15.

Fig. 6.—Solid or epithelioid type of neoplasm in one thyroid lobe. Note undifferentiated character of the solid clusters of cells separated by strands of connective tissue and the thin-walled, colloid-containing vesicles scattered in the new growth (*arrows*). This neoplasm was adjacent to the parathyroid gland. Female rat (1 month old) given propylthiouracil for 14 months. Hematoxylin and eosin; × 120.




Figures 7-9

*(See legends on opposite page)*

A distinctly different type of thyroid neoplasm is shown in Figure 6. This is a solid or epithelioid type which is undifferentiated or anaplastic. It was composed of a compact mass of cell clusters separated by delicate strands of connective tissue, with large polyhedral-shaped cells that stained poorly with the acid stains. Their nuclei were only moderately stained with basic dyes. Few of these tumor cells were arranged in the form of acini. Those present were thin-walled and made up of flattened cuboidal epithelial cells (*arrows*). Such vesicles contained a strongly acidophilic colloid and were scattered throughout the neoplasm. Mitoses were conspicuous.

Thin-walled ultimobranchial cysts were found in but two specimens of this group. In one thyroid the cyst was lined in part by simple columnar epithelium and in part by stratified squamous epithelium. There was no evidence to show that these neoplasms took origin from existing ultimobranchial cysts. Nevertheless, many new growths occupied a position which could readily be interpreted as comprising, in part, follicles derived from ultimobranchial tissue. Such a concept is supported by instances of bilateral neoplasia, where the tumors frequently occupied comparable positions within each lateral lobe, less so in rats, where multiple neoplasms developed in a single lobe.

GROUP 4 (Age 5 Months).—These nine animals were fed the experimental diet for an average period of 11 months (Table 2). The thyroid parenchyma of this group showed histological changes like those in animals of Group 3. However, the engorgement of blood vessels was not as prominent, and mitotic figures were relatively scarce for such a long period of stimulation. Five rats exhibited thyroid neoplasia. In four animals the predominating type again was that of the cystadenoma (44%). Only one rat showed the solid anaplastic type of neoplasm.

Three rats possessed single bilateral adenomata (Table 2). In two additional animals multiple tumors within a single lobe were also encountered, but only one thyroid demonstrated an early neoplasm. This occurred after seven and one-half months of treatment (Table 3). The remaining tumors were all relatively mature growths, the largest encountered in the entire study.

A thyroid lobe with two relatively mature cystadenomata is illustrated in Figure 8. The larger neoplasm lies in the center of the gland and may have been the older of the two (*a*). It is possible, also, that this new growth has been derived from ultimobranchial tissue. The smaller tumor, which is peripheral in position (*b*), exhibited a greater degree of papillary development and was probably the more rapidly growing neoplasm. The parathyroid gland is illustrated (*c*).

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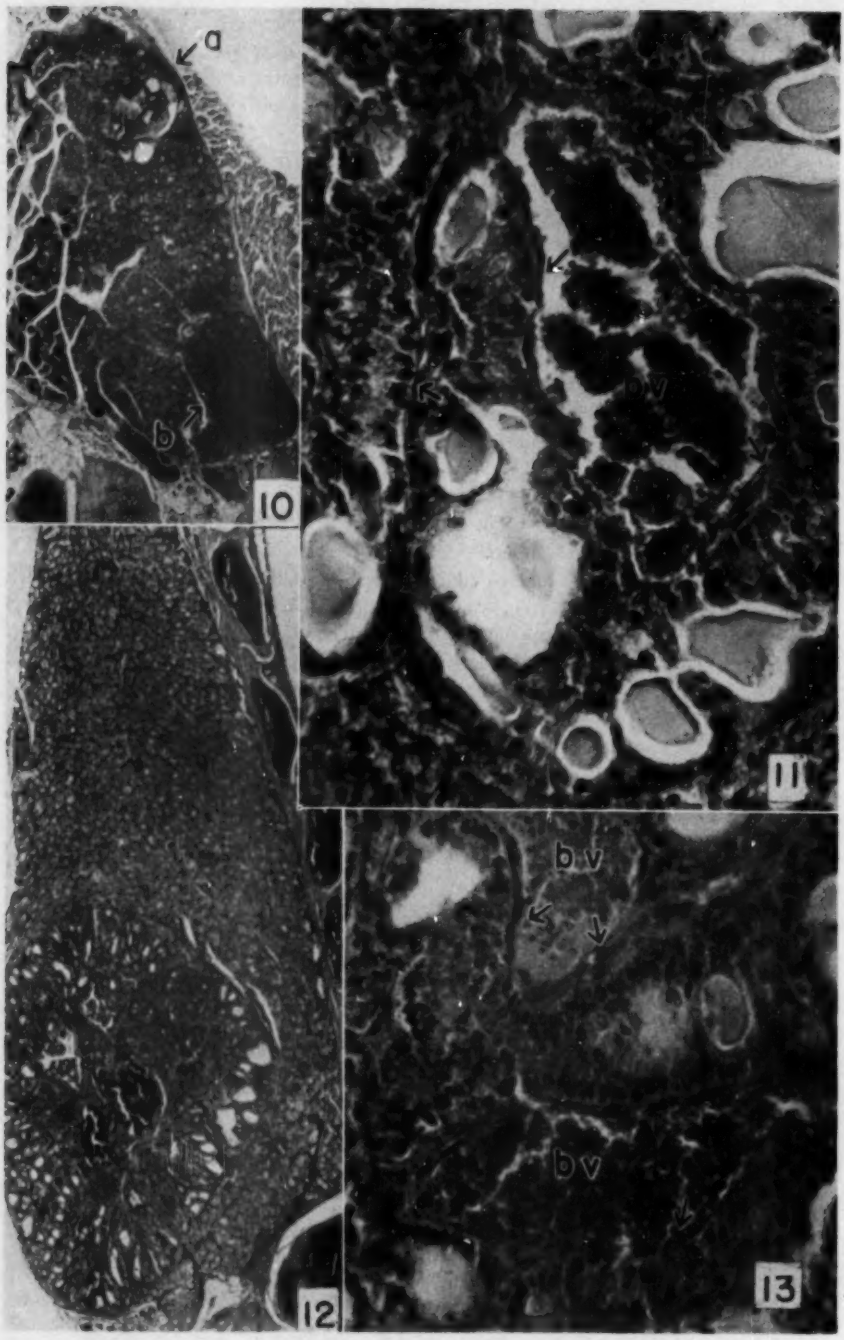
EXPLANATION OF FIGURES 7-9

Fig. 7.—Section through small cystadenoma in thyroid of female rat (2 months old) given propylthiouracil for 13 months. Note the cell-rich, densely staining character of this neoplasm and its papilliferous nature. A similar neoplasm was also present in the opposite lobe. Hematoxylin and eosin; × 120.

Fig. 8.—Thyroid lobe with relatively mature multiple adenomata. Note the larger centrally located tumor (*a*) and the smaller one showing a greater degree of papillary formation (*b*). The parathyroid gland is indicated (*c*). Similar highly vascular and multiple neoplasms were also found in the opposite thyroid lobe. Blood vessel is indicated by *bv*. Female rat (5 months old) treated with propylthiouracil for 12½ months. Hematoxylin and eosin; × 45.

Fig. 9.—Section through small papillary cystadenoma in thyroid of female rat (9 months old) maintained on propylthiouracil for two and one-half months. This neoplasm was also located centrally in plane of section of the parathyroid gland. Hematoxylin and eosin; × 130.




Figures 10-13

*(See legends on opposite page)*

Figure 12 demonstrates the largest tumor found in this study. It measured 4 mm. in its greatest diameter and was located adjacent to the parathyroid gland (not illustrated). Like others in this group, it appeared to be a very rapidly growing tumor but showed no predilection for the formation of papillary processes. Figure 13 represents a similar neoplasm which, however, was incipient. Although the densely staining cell-rich cords appear to be forming small alveoli here (*arrows*), mitotic figures could not be readily identified. It is possible such neoplasms are developing by a process of amitotic division. The engorgement of blood vessels in this Figure suggests that a more than adequate blood supply was present.

Only two animals in this group showed ultimobranchial cysts. One cystic remnant accompanied a thyroid neoplasm. While the evidence from this study has been interpreted as supporting the concept that some of these hyperchromatic adenomata originate in ultimobranchial tissue, the data at present are largely circumstantial.

## COMMENT

Diverse types of tumors recently have been induced in the thyroid gland of the laboratory rat by various methods of procedure.[19] Observations in the present study, nevertheless, indicate that prolonged stimulation of the thyroid by a goitrogen alone, in this instance propylthiouracil, can result in the predominance of a particular type of thyroid neoplasia. This type is essentially that of the hyperchromatic adenoma, which can become cystic and may be papillary. Such neoplasms occurred in approximately 42% of all the experimental animals. A second type, that of an undifferentiated or anaplastic solid neoplasm, occurred in approximately only 8% of the rats. These two kinds of neoplasia were not found within the same thyroid gland, and there was no histological evidence to indicate that either type was malignant. Unlike results obtained by Money,[9] the experimental animals under discussion did not show 100% thyroid neoplasia. However, Money used adult rats only and maintained them on a goitrogen (thiouracil) for as long as 16 months.

Why hyperchromatic adenomata should develop to the exclusion of large numbers of the undifferentiated type of neoplasm is not clear. Certain other types,

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EXPLANATION OF FIGURES 10-13

Fig. 10.—Small cystadenoma located at periphery of thyroid lobe (*a*), somewhat removed both from a central position and from the parathyroid gland (*b*). Tumor highly vascularized. Female rat (9 months old) given propylthiouracil for six and one-half months. Hematoxylin and eosin; × 30.

Fig. 11.—High-power magnification of tumor in Figure 10, showing densely staining, rapidly growing cords of cells which produce small alveoli (*arrows*). Note engorged blood vessel in center of field (*bv*). Hematoxylin and eosin; × 100.

Fig. 12.—Relatively mature thyroid adenoma located at periphery of thyroid but adjacent to the parathyroid gland (not illustrated). Opposite thyroid lobe showed multiple neoplasms. This tumor was rapidly growing but showed few papillary processes. Female rat (6 months old) given propylthiouracil for 12 months. Hematoxylin and eosin; × 45.

Fig. 13.—Incipient thyroid adenoma. Note densely staining, cell-rich cords representing this neoplasm (*arrows*). This new growth was closely related to the parathyroid gland. It shows the rapidly growing character of a tumor such as that illustrated in Figure 12. Female rat (5 months old) maintained on propylthiouracil for seven and one-half months. Note vascularity of this neoplasm as illustrated by the two engorged blood vessels (*bv*). Hematoxylin and eosin; × 100.

19. Griesbach, Kennedy, and Purves.[7] Money and Rawson.[10] Bielschowsky.[11] Money and Rawson.[14] Van Dyke.[15]

which may be intermediate neoplasms, also have been described by some investigators. It is important to note that this continuous stimulation of the thyroid, particularly in older animals, resulted in a predominance of adenomata which were completely unlike the anaplastic growths produced under other experimental conditions.[15] It should be emphasized, moreover, that these prevailing neoplasms frequently occur spontaneously in thyroids of very old rats [5] and that it is not unusual for them to show considerable degenerative phenomena. It is possible that the undifferentiated new growths may be a precursor in the development of some hyperchromatic adenomata (unpublished data).

Presumably in both the aged thyroid gland and those thyroids subjected to prolonged experimental stimulation, there would be numerous cells undergoing retrogression. This is suggested, perhaps, by the pyknotic character of the tumor cells and by their apparent amitotic method of reproduction. It is interesting to speculate that such cystic adenomata may have arisen following persistent stimulation of certain cells that have undergone partial degeneration.

Using propylthiouracil as the sole stimulating influence indicates that age is an important factor in the development of induced thyroid neoplasms in the rat. Not only did thyroid tumors occur more quickly in older experimental animals (within two months) but also they developed in greater numbers in older rats than in younger rats during approximately the same induction time (Tables 2 and 3). However, young rats will also produce large numbers of experimental thyroid tumors but require substantially longer periods of stimulation than do older rats. It is interesting to note, too, that the total average age of animals in Groups 2 and 3 is nearly the same. The results are comparable in these two groups. As in Group 3, the young adult rats in Group 4 also produced a high percentage of neoplasms after treatment for almost one year. It is quite possible that, if these adult rats had been given propylthiouracil for a longer period of time, a much higher percentage of tumors would have been encountered, perhaps equivalent to or even greater than that in the younger animals of Group 3.

The incidence of bilateral thyroid neoplasia and multiple tumors within one lobe seems to be correlated with prolonged administration of the goitrogen. This is illustrated in Groups 3 and 4 (Tables 2 and 3). However, multiple neoplasia appear to be more prevalent in thyroids of older animals.

The occurrence of many young cystadenomata in these experimental animals within the limits of time of the oldest control group leaves little doubt that such tumors have been induced by the influence of excessive thyroid stimulation. It is concluded, therefore, that cystadenomata are prone to develop in older experimental animals and can occur in young rats only after prolonged goitrogen administration.

Certain observations by other investigators concerning the role of age in induced tumorigenesis may be mentioned. According to Kaplan,[20] the leukemogenic action of irradiation declines following the period of sexual maturity. Likewise, the incidence of urethan-induced lung tumors apparently declined as the age of the animal increased at the time of exposure to the carcinogen (Rogers [21]). Silberberg

20. Kaplan, H. S.: Influence of Age on Susceptibility of Mice to the Development of Lymphoid Tumors After Irradiation, J. Nat. Cancer Inst. **9**:55-56, 1948.

21. Rogers, S.: Age of the Host and Other Factors Affecting the Production with Urethane of Pulmonary Adenomas in Mice, J. Exper. Med. **93**:427-449, 1951.

and Silberberg [22] state that there is a decreasing susceptibility of tissues to growth stimuli with advancing age.

The results of the present study, however, do not support this concept for the development of nodular tumors (cystadenomata) in the thyroid gland of rats. Nevertheless, insofar as hyperplasia and growth of the thyroid as a whole are concerned, this would appear to be true. In animals possessing no neoplasms, the general thyroid parenchyma in younger rats responded to the goitrogen more readily and to a greater degree than in older animals. In the latter animals there was an observed diminution on the part of thyroid tissue to undergo continued proliferation. Since the age of maximum susceptibility to stimuli varies with the kind of tissue on which the stimulus acts (Silberberg and Silberberg [22]), it is entirely possible that some neoplasms in the mammalian thyroid gland have an origin unlike that in many other organs.

Mention should be made of the accumulating evidence which points toward the ultimobranchial tissue as the source of these thyroid neoplasms, possibly from metaplastic lesions (cysts) composed of stratified squamous epithelium. It is possible that, as in the case of spontaneous cystadenomata in senile rats, such epithelial cysts have contributed to the formation of these hyperchromatic adenomata. However, the morphological details of their development may have been obscured by the persistent parenchymatous hyperplasia. According to Steiner [23] metaplastic lesions are the source of neoplasms in certain organs.

Since chronic vitamin A deficiency will usually induce metaplasia (cysts) in ultimobranchial tissue of the rat thyroid,[17] studies utilizing a goitrogen during such vitamin deficiency may clarify the part played by this tissue during thyroid tumorigenesis. Such studies are now in progress. In this study the location of bilateral thyroid neoplasms near the center of each lobe, in company with a parathyroid gland, also suggests the origin of such tumors from ultimobranchial tissue. Since Gorbman and Evans [24] have described a centrifugal growth pattern of differentiation in the thyroid of fetal rats, multiple neoplasms might be explained, in part, on the basis of dissemination of ultimobranchial tissue following prolonged stimulation. This would be more prone to occur in aging animals and would help explain certain peripheral tumors. Furthermore, in birds the ultimobranchial body does not become incorporated within the thyroid gland.[25] Cockerels studied after treatment with thiouracil for 24 months showed only diffuse hyperplasia but no neoplastic changes.[10]

If young tissues are more susceptible to growth stimuli, it should be pointed out that ultimobranchial tissue is a labile tissue with attributes unlike those of adult thyroid tissue and that it can also occur in the human thyroid gland. This accessory thyroidal component is essentially an undifferentiated tissue in which the thyroid-like characteristics apparently are dependent upon induction during fetal develop-

22. Silberberg, M., and Silberberg, R.: Relation Between Age and Tissue Response to Stimuli: A Review, J. Gerontol. **7**:399-404, 1952.

23. Steiner, P. E.: Human Significance of Experimental Carcinogenesis, A. M. A. Arch. Path. **55**:227-244, 1953.

24. Gorbman, A., and Evans, H. M.: Beginning of Function in Thyroid of Fetal Rat, Endocrinology **32**:113-115, 1943.

25. Dudley, J.: The Development of the Ultimobranchial Body of the Fowl Gallus Domesticus, Am. J. Anat. **71**:65-98, 1942.

ment by a growing functional thyroid gland.[5] With age and subsequent diminution in thyroid activity, its ability to function in iodine metabolism may be altered. As a result, possibly, of metaplasia and/or following persistent thyrotropic hormone stimulation, this indifferent mass may produce highly vascular compensatory tumors of a particular kind. Under the present experimental conditions these would appear to be hyperchromatic (papillary) cystadenomata. Similar aberrant growths in the human thyroid gland, perhaps of ultimobranchial tissue origin, may also be the result of such prolonged stimulation.

SUMMARY

A study has been made of the sole influence of a goitrogen, propylthiouracil, on production of thyroid tumors during various age periods in female Wistar rats. Spontaneous thyroid neoplasia is rare in these animals. However, experimental thyroid tumorigenesis occurs more quickly and in a higher percentage of older rats than in young rats. Hyperchromatic (papillary) cystadenomata are more prevalent following the stimulating influence of a goitrogen alone, especially after prolonged administration. Circumstantial evidence indicates that these tumors are derived, at least in some instances, from the ultimobranchial tissue component of the thyroid gland. This tissue is prone to form spontaneous cystadenomata in thyroids of senile rats. A similar origin for certain human thyroid tumors following continuous stimulation has been suggested.

# EFFECT OF CORTISONE AND PARTIAL STARVATION ON LIVER REGENERATION

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ST. LOUIS

IT HAS been established that cortisone inhibits regeneration and proliferation of connective tissues.[1] Much less information regarding the effect of cortisone on the regeneration of parenchymatous organs is available.

Hall and Hall [2] reported that cortisone enhances compensatory hypertrophy of the remaining kidney after unilateral nephrectomy in rats. With regard to the liver it has been reported that the intact liver increases in size during the administration of cortisone for a short time in the rat,[3] rabbit,[4] and mouse.[5] The concentration of glycogen increases above normal; however, glycogen accounts for only a small part of the increased liver mass. The protein, fat, and water concentrations are not significantly affected. The hepatic cells have been shown to increase in size, but no increase in mitotic activity has been reported. Since investigations have failed to demonstrate a significant increase in any particular cellular constitutent or in the

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From the Department of Pathology, Washington University School of Medicine, and the Laboratory of the Barnard Free Skin and Cancer Hospital.

1. Ragan, C.; Howes, E. L.; Plotz, C. M.; Meyer, K.; Blunt, J. W., and Lattes, R.: The Effect of ACTH and Cortisone on Connective Tissue, Bull. New York Acad. Med. **26**:251, 1950. Alrich, E. M.; Carter, J. P., and Lehman, E. P.: The Effect of ACTH and Cortisone on Wound Healing, Ann. Surg. **133**:783, 1951. Bangham, A. D.: The Effect of Cortisone on Wound Healing, Brit. J. Exper. Path. **32**:77, 1951.

2. Hall, C. E., and Hall, O.: Growth Effects of Desoxycorticosterone and Cortisone with Special Reference to Compensatory Hypertrophy, Proc. Soc. Exper. Biol. & Med. **79**:536, 1952.

3. Venning, E. H.; Kazmin, V. E., and Bell, J. C.: Biological Assay of Adrenal Corticoids, Endocrinology **38**:79, 1946. Wang, F. C., and Verzar, F.: Comparison Between Glycogenetic Property of Desoxycorticosterone, 11-Dehydro-17-Hydroxy-Corticosterone (Compound E) and Adrenal Cortical Extract, Am. J. Physiol. **159**:263, 1949.

4. Timiras, P. S., and Koch, P.: Morphological and Chemical Changes Elicited in the Liver of the Rabbit by Cortisone and Desoxycorticosterone Acetate, Anat. Rec. **113**:349, 1952. Reinecke, R. M., and Kendall, E. C.: A Comparison of the Influence of Some Crystalline Hormones of the Adrenal Cortex on the Deposition of Glycogen in the Liver, Endocrinology **32**:505, 1943.

5. Roberts, K. B.; Florey, H. W., and Joklik, W. K.: The Influence of Cortisone on Cell Division, Quart. J. Exper. Physiol. **37**:239, 1952.

number of hepatic cells, this suggests that the increase in weight of the liver is due to an increase in all the major constitutents of the cytoplasm rather than to the deposition of a single substance or to multiplication of hepatic cells. After more prolonged administration of cortisone for a period of six weeks, the liver in intact rats does not change in size; also, there is no effect on the size of the heart, pituitary, and testis.[6]

Roberts, Florey, and Joklik[5] have recently studied the effect of large doses of cortisone on liver regeneration in mice after partial hepatectomy. At two days after partial hepatectomy, mitoses were greatly inhibited in the cortisone-treated group, as compared with a control group; however, at six days no difference in number of mitoses was apparent. No differences were found in mitotic activity of the intestinal epithelium at any time. The increase in liver weight after partial hepatectomy was essentially the same in cortisone-treated and in control mice. The increase in liver weight of the cortisone groups was largely due to accumulation of glycogen and fat.

The present experiments were designed to investigate the possibility of a specific effect of cortisone on regeneration of the liver in the rat, a species in which liver

*Treatment of Experimental and Control Groups*

| Group No. | No. of Rats | Operative Procedure | Cortisone | Diet * |
|---|---|---|---|---|
| I | 20 | Partial hepatectomy | 0 | Normal |
| II | 20 | Partial hepatectomy | x | Normal |
| III | 20 | Partial hepatectomy | 0 | Restricted |
| IV | 20 | Laparotomy | 0 | Restricted |
| V | 20 | Laparotomy | x | Normal |
| VI | 9 | Laparotomy | 0 | Restricted and normal |

* All groups received rat pellets of the Ralston Purina Company, St. Louis.

regeneration has been thoroughly studied under a variety of conditions.[7] Also the effects of cortisone and partial starvation on liver regeneration were compared, as well as the effects of cortisone in the intact and regenerating liver.

## MATERIAL AND METHODS

Experimental procedures are summarized in the Table. Healthy male albino rats weighing between 125 and 225 gm. were used.

The body weight of each animal was recorded daily. All animals had free access to water throughout the experiment. Animals in Groups I, II, and V were fed ad libitum. The diet given to animals in Groups III and IV was restricted to effect a body-weight loss equal to that sustained by the animals in Group II. This was done by varying the number of pellets given on the basis of the weight changes from the previous day. By selection of animals from the total group, it was possible to kill animals with an average weight loss not differing significantly from that of the animals killed on that day in Group II. The animals in Group VI were made

6. Winter, C. A.; Silber, R. H., and Stoerk, H. C.: Production of Reversible Hyperadrenocortinism in Rats by Prolonged Administration of Cortisone, Endocrinology **47**:60, 1950.

7. Brues, A. M.; Drury, D. R., and Brues, M. C.: A Quanitative Study of Cell Growth in Regenerating Liver, Arch. Path. **22**:658, 1936. Brues, A. M., and Marble, B. B.: An Analysis of Mitosis in Liver Restoration, J. Exper. Med. **65**:15, 1937. Wenneker, A. S., and Sussman, N.: Regeneration of Liver Tissue Following Partial Hepatectomy in Parabiotic Rats, Proc. Soc. Exper. Biol. & Med. **76**:683, 1951.

to lose weight by the same manner during the first 6 days and were allowed to gain weight during the subsequent six-day period to parallel the body-weight changes in Group I. All animals were housed in air-conditioned quarters throughout the experiment. All animals were subjected to ether anesthesia. Partial hepatectomy was performed by removing two lobes of the liver by the method of Higgins and Anderson[7] with minor modifications. The weight of liver removed by this method was determined on a group of nine animals not included in the Table. After partial hepatectomy, these animals were killed and the remaining liver was removed. By this method it was determined that 64.9% ± 1.2% was removed by partial hepatectomy. Sixty-five per cent has been used as the value in all calculations. The quantity of liver regenerated is defined as the per cent of liver which the animal has replaced of the amount removed. Thus, the 35% remaining in the rat at the time of partial hepatectomy is subtracted from the total amount of liver at the time of autopsy; the remainder is expressed as a per cent of the amount removed at the time of partial hepatectomy. As will be shown, additional specific corrections are necessary. Administration of cortisone was begun three days before operation and continued until the animals were killed; daily subcutaneous injections of 0.1 cc. cortisone acetate per 100 gm. of body weight were used. This dose has been found effective in inhibition of connective tissue deposition in the rat.[8]

Five animals from each of the Groups I to V were killed, with ether on days 2, 4, 8, and 12 after operative procedures, with few exceptions. Animals from Group VI were killed at 8 and 12 days after laparotomy.

The purpose of Groups I, II, and III is apparent. Groups IV, V, and VI were designed to control the effects of changes in the body weight and of cortisone on the weight of the liver. The initial liver weight of the nonhepatectomized groups (IV, V, and VI) has been considered to be the same as the liver weight of hepatectomized animals of similar average body weight.

A random sample (approximately 100 mg.) of normal or regenerated liver was accurately weighed for chemical determinations. Water content was determined by macerating the sample in an alcohol-ether mixture, drying to constant weight at 68 C., and determining the difference from the initial weight. Fat content was determined on this dried sample by extracting to constant weight with a mixture of equal parts of absolute alcohol and ether and is represented by the weight difference after this procedure. The protein content was determined by the method of Lowry and co-workers [9] after homogenization of the remainder with trichloroacetic acid.

Tissue from two different areas of regenerated liver was immediately fixed in Bouin's fluid; hematoxylin and eosin-stained sections were prepared. The following data were obtained from these slides: (*a*) average number of hepatic parenchymal cells in a high-power area, 10 microscopic fields per animal being counted; (*b*) total number of mitoses in hepatic cells per 100 high-power fields; (*c*) Chalkley [10] counts (300 nuclear "hits" per animal) to determine the ratio of total volume occupied by hepatic nuclei, hepatic cytoplasm, and sinusoids in the livers of animals killed at 12 days. From these measurements the following information was obtained as ratios rather than as absolute values:

1. Total number of regenerated cells—average number in high-power field times weight of liver regenerated (after appropriate corrections).

2. Mitotic activity—total number of mitoses divided by average number of cells per high-power area.

3. Average volume of hepatic cells—(nuclear "hits" plus cytoplasmic "hits" divided by nuclear "hits" plus cytoplasmic "hits" plus sinusoidal "hits") divided by average number hepatic cells per high-power field.

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8. Cortisone used was generously supplied by Merck and Company, Inc., Rahway, N. J.

9. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L., and Randall, R. J.: Protein Measurement with the Folin Phenol Reagent, J. Biol. Chem. **193**:265, 1951.

10. Chalkley, H. W.: Method for the Quantitative Morphologic Analysis of Tissues, J. Nat. Cancer Inst. **4**:47, 1943.

RESULTS

The changes in body weight of partially hepatectomized animals (Groups I, II, and III) are shown in Chart 1. The mass of liver regenerated by these three groups is shown in Chart 2. However, it is desirable to make an adjustment for the effect

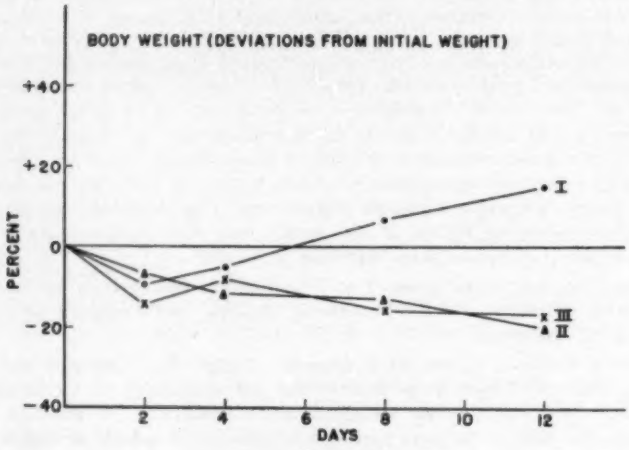


Chart 1

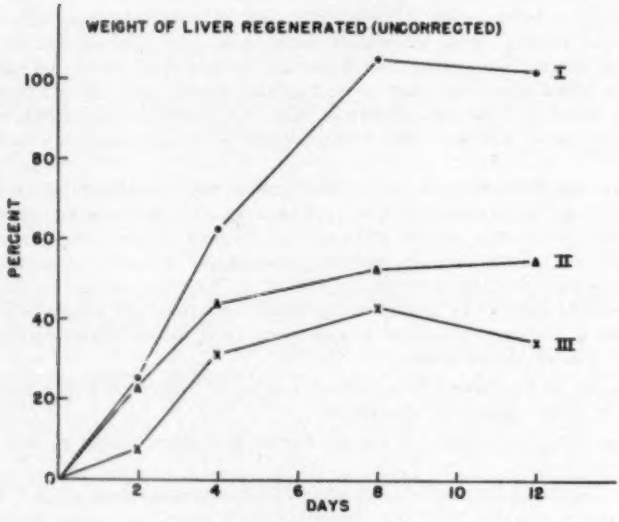


Chart 2

of change in body weight on liver weight [11] from a sham-operated control group (Groups IV and VI) if the specific effect on regeneration is to be determined;

11. Jackson, C. M.: Effects of Acute and Chronic Inanition upon the Relative Weights of the Various Organs and Systems of Adult Albino Rats, Am. J. Anat. **18**:75, 1915. McLennan, C. E., and Jackson, C. M.: Weights of Various Organs in the Adult Rat After Inanition With or Without Dietary Accessories, Arch. Path. **15**:636, 1933.

also, a similar adjustment must be made for the effect of cortisone on liver weight in sham-operated animals (Group V). The magnitude of these effects is indicated in Chart 3. Corrections were made as follows: The per cent of liver-weight loss for a given body-weight loss was determined from Chart 3 and subtracted from both

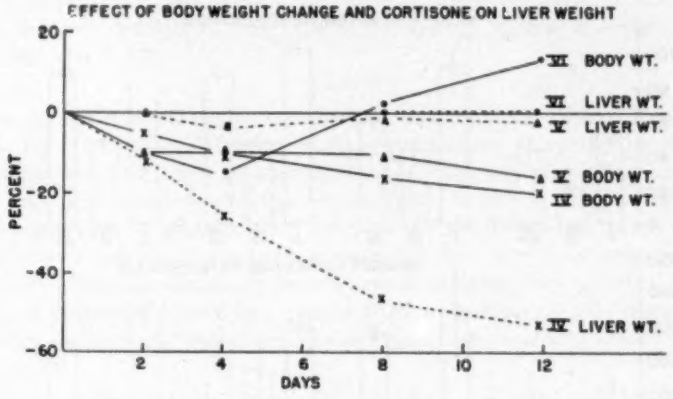


Chart 3

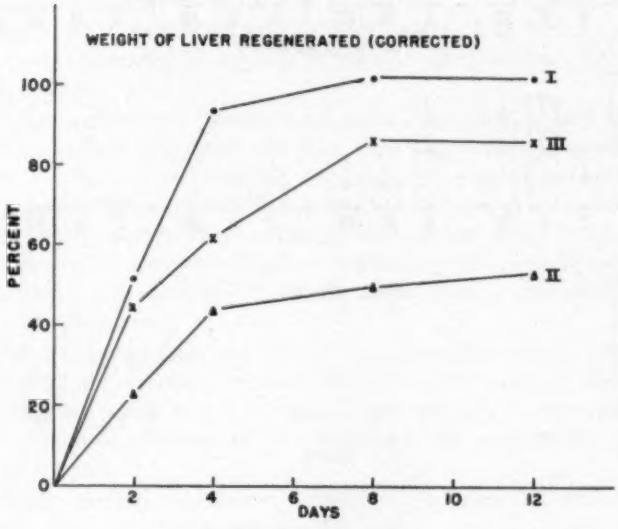


Chart 4

the amount removed and the amount left in the rat at the time of partial hepatectomy. This reduced the initial liver weight to the value that might have been expected if the liver had remained intact in the animal. At the time of autopsy the total weight of the liver was determined. The corrected weight of the amount of liver remaining in the animal after partial hepatectomy was subtracted from this value.

The remainder was considered to be the weight of liver regenerated. It is expressed as a per cent of the corrected weight of the two lobes removed at partial hepatectomy. Chart 4 indicates the "true" weight of liver regenerated after these corrections have been made. By this method of correction the weight regenerated by the partially

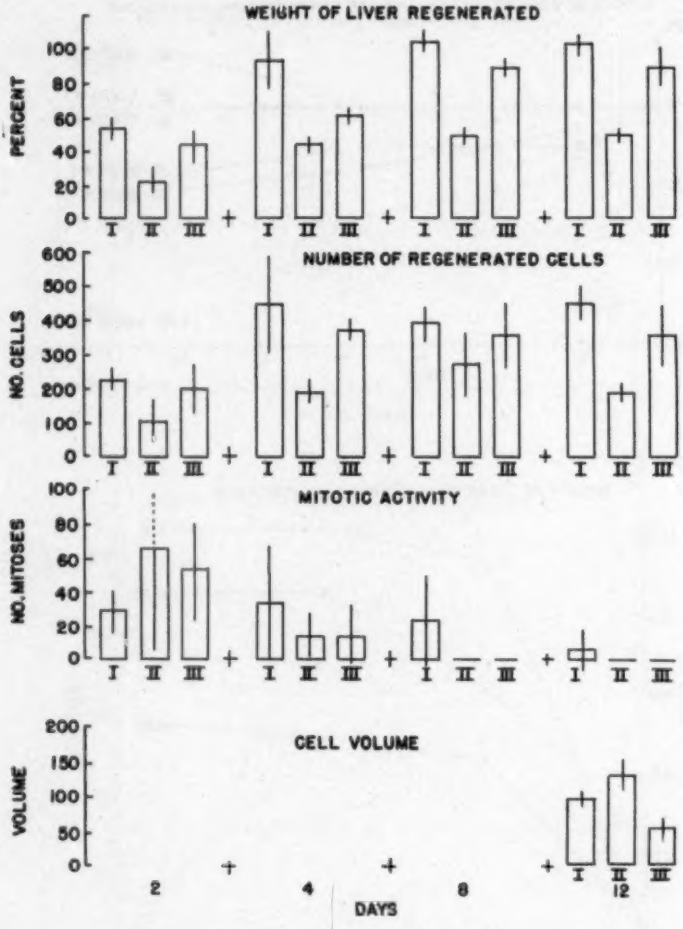


Chart 5

starved group (III) is much greater than the uncorrected weight shown in Chart 2, and the cortisone-treated animals (Group II) regenerated much less liver. In fact, the differences in liver regeneration observed between Groups I and III are not statistically significant. On the other hand, differences between Group II and normal or partially starved animals are highly significant (at the 1% level) at 4, 8, and 12 days.

The values obtained from determination of water, fat, and protein were relatively constant for normal, as well as for regenerated, liver obtained at 2, 4, 8, or 12 days from all experimental groups. Ranges for water, protein, and fat of 68 to 81%, 15 to 22%, and 3.6 to 8.6%, respectively, are within the ranges reported in the literature.[12]

The comparative values of the "true" mass of regenerated liver, of mitotic activity, of the total number of cells, and of the average volume of hepatic cells are shown in Chart 5.

## COMMENT

The results of these experiments indicate that cortisone inhibits liver regeneration in the rat, considering either weight of parenchyma or number of cells. This effect is similar to the effect on connective tissue. The inhibitory effect of cortisone on the liver is partially explained by the findings indicated in Chart 5; the number of cells regenerated by animals given cortisone is considerably less than the number regenerated by animals on a normal or restricted diet. Also there is an increase in hepatic cell volume in animals given cortisone, confirming other reports.[13] Although the weight regenerated is slightly greater than the number of cells regenerated in animals given cortisone, the larger size of hepatic cells probably accounts for this difference.

Another striking effect of cortisone is the maintenance of liver weight in animals losing body weight. It has been reported that cortisone increases the liver weight in normal rats and rabbits, and it has also been shown that body weight loss is accompanied by a marked decrease in liver weight. Our findings suggest that these two factors, operating in opposite directions, tend to maintain the liver weight at a constant level.

Our results indicate that partial starvation has no effect on liver regeneration. Brues and co-workers[7] reported that liver mass regenerated by partially starved animals was less than normal, although the number of cells regenerated was normal. The results reported here are difficult to compare with those of Brues and his group, because the method used by these authors to make adjustments for changes in body weight is obscure. The number of cells regenerated by the partially starved groups is not statistically different from that of the normal group, confirming the findings of Brues and co-workers.

The finding that fewer hepatic cells are regenerated by animals given cortisone may be clarified by the recent findings of Roberts and co-workers.[5] As noted above, these investigators found that cortisone inhibited mitoses in regenerating hepatic cells after partial hepatectomy. It is unfortunate that quantitative data are not given in their report.

Considering the data available to date, it may be postulated that cortisone inhibits liver regeneration by decreasing the number of cells regenerated through inhibition of mitotic activity. The increase in size of hepatic cells is apparently an increase in the amount of cytoplasm rather than the deposition of a single substance.

12. Li, C. H.; Simpson, M. E., and Evans, H. M.: The Influence of Growth and Adrenocorticotropic Hormones on the Fat Content of the Liver, Arch. Biochem. **23**:51, 1949.

13. Footnote 3. Footnote 4.

## SUMMARY AND CONCLUSIONS

The influence of cortisone and partial starvation on liver regeneration has been studied in the rat. The effect of cortisone on intact and regenerating livers has been compared.

The following conclusions are made:

1. Cortisone inhibits regeneration of the liver considered either as mass or as number of cells.

2. Cortisone increases the size of hepatic cells in both regenerating and normal livers.

3. The increase in size of hepatic cells with administration of cortisone is apparently due to an increase in cytoplasm; determinations of water, protein, and fat concentration did not differ significantly among any of the experimental groups.

4. Liver weight is maintained by cortisone in the presence of body-weight loss.

5. Liver regeneration is not affected by partial starvation.

# CEREBROVASCULAR SIDEROSIS IN HORSES

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VASCULAR siderosis in man has been observed in a wide variety of brain diseases. It has also been recorded as the only pathologic finding in various psychotic states and as being frequently present in the brains of healthy human beings over 30 years of age. The latter observation would, of course, tend to minimize the significance of vascular siderosis when found in diseases in which the symptoms were referable to the brain. With reference to human brains, Cobb [1] wrote in 1932:

> The deposit of iron in the walls of cerebral vessels has been shown to be so common that it may be considered within normal limits when found in the vessels of the midbrain and basal ganglia. In these regions of the brain the content of iron is very high in the parenchyma, probably most of it being in the form of pigment in the nerve cells. Chronic changes of advancing age transfer this iron to the vessel walls. In encephalitis lethargica and in fact any degeneration or inflammation this process is enhanced and iron rings may be found about the vessels of young people. In other parts of the brain, iron deposits frequently occur in the vessel walls only when there has been a hemorrhage with extravasation of red cells into the surrounding tissues.

While the above interpretation of the significance of cerebrovascular siderosis might have served to reconcile conflicting opinions on the subject, examination of the current literature showed that it had not done so. Strassman [2] was still able to state in 1949 that, because of the variety of conditions under which iron deposits were found in the brain, opinions differed regarding their diagnostic and pathologic significance. It was therefore deemed advisable to investigate the subject further, particularly in horses, where such deposits were frequently seen during routine histologic studies of brains in this laboratory. With the exception of an occasional cow brain, we have not encountered vascular siderosis in any other species. In accordance with our experience, Innes [3] stated that he had seen siderosis only in horse brains and had not encountered it in sheep or dogs, despite systematic search.

The type of iron deposit under consideration here is the so-called autogenic or tissue iron and not hematogenic iron derived from hemosiderin, which occurs in the region of cerebral hemorrhages. Autogenic deposits are thought to occur by

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1. Cobb, S., in Penfield, W.: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932, Vol. 2, p. 605.

2. Strassman, G.: Iron and Calcium Deposits in the Brain: Their Pathologic Significance, J. Neuropath. & Exper. Neurol. **8**:428-435, 1949.

3. Innes, J. R. M.: Personal communication to the author.

focal concentration of the iron present in certain regions of the brain, as described by Cobb above. When a sufficient amount of iron has been concentrated in any one site, a visible deposit results.

## LITERATURE

Two studies have been made on iron deposits in the brains of domestic animals, either of which might have been expected to indicate their diagnostic significance. However, neither of these obviated the need for further investigation.

Kikuchi,[4] in 1928, described the changes incident to advancing age in the brain of the horse. While his detailed and painstaking study was in many respects a classic, only 4 of his 23 horses were abattoir material and hence presumably healthy. Of the remaining 19 horses, 6 were affected with glanders, and the others represented a variety of diseases, including tuberculosis, purulent bronchopneumonia, hemorrhagic enteritis, peritonitis, and bacteremia. Kikuchi stressed the fact that brains were selected only from animals that showed no clinical evidence of brain disease. He then described certain changes, including siderosis, in the horse brains which increased in severity with advancing age and concluded that they were due to senility. It seems that such changes might better be studied in animals not affected with systemic disease of any kind if valid conclusions are to be drawn, since systemic disease may predispose to siderosis of the brain vessels.

Hurst [5] studied the brains of 16 horses, 1 mule, and 1 pony. He found iron and calcium deposits in the cerebral vessels of the older ones and concluded that these were attributable to senility. Since the equines in his series had died of acute disease, including brain disease (equine encephalomyelitis, "forage poisoning"), a conclusion based on such material seems questionable.

## MATERIALS AND METHODS

It was thought that if the occurrence of vascular siderosis in the brains of horses was analogous to its occurrence in man, then Kikuchi and Hurst had probably drawn correct conclusions, which required only some better material for confirmation. Accordingly, it was decided to investigate the presence of siderosis in the brains of healthy horses of various ages. Healthy horses being slaughtered for human consumption at an abattoir were selected, since these could be observed both ante and post mortem.

The horses were examined individually in the pens shortly before slaughter. They were predominantly of heavy draft type, and the majority appeared to be in good or excellent nutritional condition. Although a complete neurologic examination could not be made, all of the horses used in this study appeared healthy and showed no clinical evidence of central nervous system disease. The animals were followed through at postmortem examination, which was conducted by Dr. S. Steiber, federal inspector in charge of the abattoir. Gross lesions of various kinds were found in the thoracic and abdominal viscera of some of the animals, but only horses that were free from gross lesions were used for this study.

The horses were shot with a rifle and exsanguinated. The skulls were disarticulated and split longitudinally by the butchers. Since technical considerations made it necessary to limit the amount of brain to be sectioned, the sampling procedure was restricted to the basal ganglia. A block of tissue from this region was excised within 10 to 15 minutes post mortem and fixed

4. Kikuchi, K.: Über die Altersveränderungen am Gehirn des Pferdes, Arch. wissensch. u. prakt. Tierh. **58**:541-573, 1928.

5. Hurst, E. W.: Calcification in the Brains of Equidae and Bovidae, Am. J. Path. **10**:795-798, 1934.

in 10% neutral aqueous formalin. The rifle bullets were aimed at the medulla oblongata. The aim was accurate and produced a discrete lesion in the brain stem; we encountered no blood clots or torn vessels in the region of the basal ganglia.

In addition to the 19 brains that were obtained at the abattoir, 6 brains were obtained from horses that were submitted for euthanasia because of localized disease of the limbs (chronic lameness). In the latter horses, the existence of systemic disease was ruled out by careful post-mortem examination. Two brains from animals that did not meet the criteria of absence of systemic diseases were also examined. One was from a stillborn foal that was three weeks premature. The other was from a 6-week-old foal that died of thrombosis of the posterior vena cava, apparently as a consequence of navel infection. These brains were examined in an attempt to fix the lowest age limit at which siderosis could be found, but no siderosis was seen in either one. They are excluded from the discussion that follows.

*Horse Brains Examined for Vascular Siderosis* *

| No. | Age, Yr.† | Sex | Siderosis |
|---|---|---|---|
| 1 | 7 | M | + |
| 2 | 9 | F | + |
| 3 | 25 to 30 | F | + |
| 4 | 8 | M | − |
| 5 | 6 | F | + |
| 6 | 6 | M | + |
| 7 | 8 | M | + |
| 8 | A | M | + |
| 9 | A | F | − |
| 10 | A | M | + |
| 11 | A | F | − |
| 12 | A | F | + |
| 13 | A | M | + |
| 14 | A | M | + |
| 15 | A | F | + |
| 16 | A | M | + |
| 17 | A | F | − |
| 18 | A | M | − |
| 19 | 6 | F | − |
| 20 | 11 | F | − |
| 21 | 14 | F | − |
| 22 | 11 | M | + |
| 23 | 5 | M | − |
| 24 | 20 | M | + |
| 25 | 15 | M | − |
| Totals | | | 15+<br>10− |

* Horses 1 to 19 are abattoir animals; their ages were estimated by examination of the teeth. Horses 20 to 25 were clinic animals; their exact ages were available in the case records.
† A indicates aged animals.

The Table shows the source, age, and sex of the horses supplying the brain material. In only one instance, No. 3, did we attempt to estimate the age of any animal over 9 years old. Those that were considered to be above that age were simply recorded as A (aged). They probably varied between 10 and 20 years of age. We were unable to obtain any animals under 6 years of age at the abattoir.

Hematoxylin and eosin stain was used to find the iron deposits and a modified Perls's iron stain [6] on the duplicate sections to identify the deposits. A brain was recorded as +, even if only one vessel was found to be affected. No attempt was made to work out a quantitative grading system to indicate the extent of involvement. It was realized that, with the sampling technique that was employed, some of the brains in which no iron was found probably would have yielded iron if more sections had been taken.

6. Highman, B.: New Modification of Perl's Reaction for Hemosiderin in Tissues, Arch. Path. **33**:937-938, 1942.

COMMENT

The iron deposits were found to occur both in vessel walls and extravascularly. Extravascular deposits were only seen in areas where there were a number of affected vessels, however (Fig. 1). The heaviest deposits were in the globus pallidus, but siderosis was also found in the adjacent internal capsule and in the caudate nucleus. The two brains in which the most extensive siderosis was found were from the abattoir material, Nos. 3 and 7. Both arteries and veins were affected.

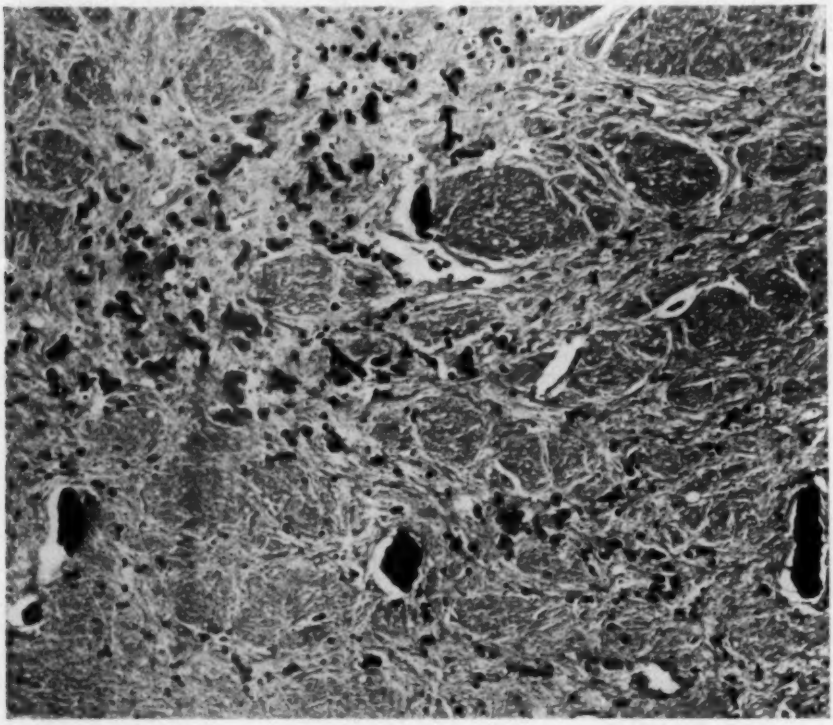

Fig. 1.—Vascular siderosis in globus pallidus of horse. The numerous small deposits adjacent to the vessels are extravascular iron; hematoxylin and eosin.

Various stages of ferrugination could be observed in the vessels. In one of the earliest, the deposits were present in the tunica externa in the form of tiny particles. In intermediate stages some of these had coalesced to produce solid opaque plaques, and the process was extending toward the lumen of the vessel (Fig. 2A). In the last stages there were many instances of almost complete occlusion of the vessels (Fig. 2B). This was not an apparent occlusion due to agonal contraction of the arteriolar muscle fibers. One might reasonably expect to find ischemic changes in the neighborhood of such vessels, but none were seen. Chronic passive congestion was not observed in adjacent vessels not involved by siderosis. It would appear that the collateral circulation to and from the basal ganglia is sufficient to prevent such sequelae.

The material does not represent a complete age distribution, and most of the ages had to be determined by estimation. In view of this, it does not lend itself to correlation of the presence of iron deposits with advancing chronological age.

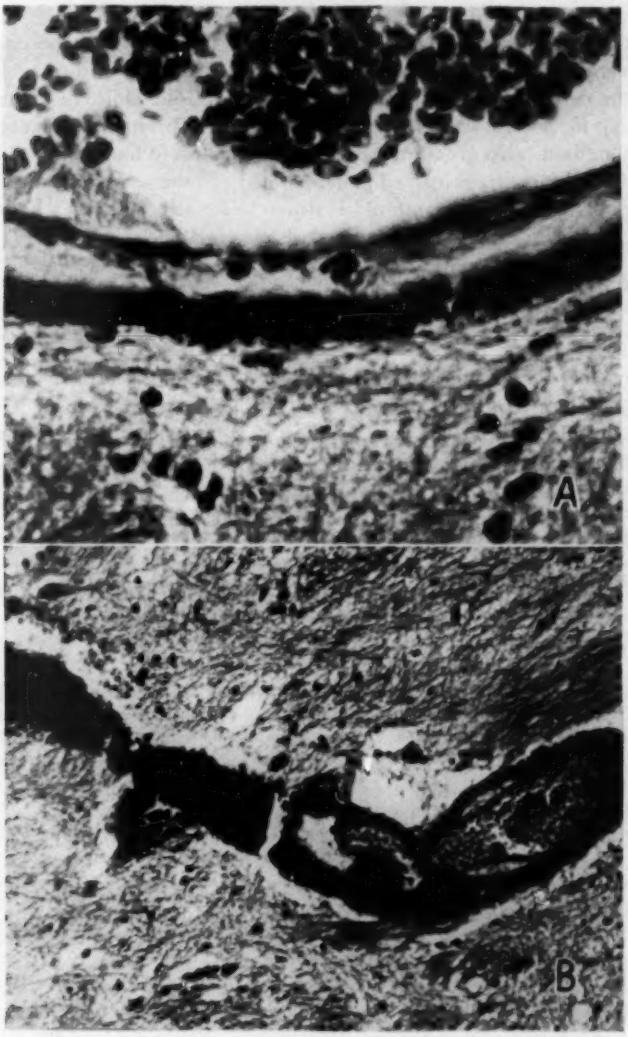


Fig. 2.—*A*, vascular siderosis in globus pallidus of horse. High-power view of iron in a finely particulate form peripheral to the solid deposits; hematoxylin and eosin. *B*, vascular siderosis in globus pallidus of horse. Partial occlusion of the vessel lumen by nodular-appearing iron deposits; hematoxylin and eosin.

However, it does show that among horses over 6 years of age, vascular siderosis can be detected in the brain with the simplest of sampling techniques. It was present in 15 of the 25 brains in this series of the animals 6 years old and older. The likeli-

hood is that a more elaborate sampling technique would have resulted in detecting siderosis in a higher proportion of brains of this same series.

Since most of the material was selected from healthy animals being butchered for human consumption in an inspected abattoir, it forms a valid basis on which to draw conclusions.

No systematic search was made for siderosis in the brains of other species, but two cases were found in cattle during the course of this study. In one instance the vessels of the cerebellum were involved in a one-year-old Holstein heifer. It had been suffering for several weeks from anorexia and was presented for autopsy in an emaciated condition. No gross or microscopic lesions were found post mortem, and the reason for its anorexia remained undetermined. In the second instance the basal ganglia were involved in a 6-month-old Holstein heifer affected with a brain abscess. The details of this case have been described elsewhere.[7] These two cases support the contention that siderosis may be present in young persons under the influence of generalized disease or of disease localized in the brain.

## SUMMARY

Vascular siderosis was found in the brain of 15 out of 25 healthy horses over 6 years of age. Since it is so commonly found in apparently healthy older animals, its presence would have little or no diagnostic significance in animals presenting clinical symptoms of brain disease.

The Eastern Packing Co., Newark, N. J., cooperated in providing the horse-brain specimens. Dr. J. W. Grogan assisted in the clinical examination and in estimating the ages of the horses at the abattoir.

7. Blood, D. C., and Saunders, L. Z.: Cerebral Abscess in a Calf, Cornell Vet. **40**:336-345, 1950.

# Case Reports

## ARGENTAFFIN CARCINOMA (CARCINOID) OF MECKEL'S DIVERTICULUM WITH METASTASIS TO LIVER

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FREQUENT reports and adequate recent review articles on argentaffin carcinoma, or "carcinoid," (Foreman,[1] Ariel,[2] and Willis[3]) have appeared in the literature. Only seven cases have been recorded in which Meckel's diverticulum was the primary site (Price,[4] Hertzog and Carlson,[5] Ashworth and Wallace,[6] Collins,[7] Pautler and Scotti,[8] Stewart and Taylor[9]). In none of these were metastases present, so the following case is being reported.

### REPORT OF A CASE

In the autopsy of a 58-year-old white man with advanced arteriosclerotic and hypertensive cardiovascular-renal disease, the usual gross and microscopic findings of arteriolonephrosclerosis and coronary sclerosis were found. The liver, which weighed 2,000 gm. and showed chronic passive congestion, contained a discrete firm yellow-gray nodule 0.5 cm. in diameter. Microscopically (Fig. 1*A*) this tumor was composed of sheets of densely packed polyhedral cells with light eosinophilic cytoplasm and small round dark basophilic nuclei. The cells at the periphery of the neoplasm were columnar and arranged in a radial fashion. The stroma consisted of loose vascular connective tissue. A diagnosis of argentaffin carcinoma (carcinoid) was made.

Examination of the appendix, rectum, and small bowel, exclusive of a Meckel diverticulum, revealed no primary site for the tumor. The lungs contained neither a bronchial adenoma nor metastases.

A Meckel diverticulum was found 40 cm. from the ileocecal junction. It measured 3 cm. in length and 1 cm. in diameter. The serosal surface was smooth and glistening. On section, a 0.5 cm. yellow-gray nodule was found in the submucosa at the apex of the diverticulum. It

From the Department of Pathology, Veterans Administration Hospital.

1. Foreman, R. C.: Carcinoid Tumors: A Report of 38 Cases, Ann. Surg. **136**:838, 1952.

2. Ariel, I. M.: Argentaffin (Carcinoid) Tumors of the Small Intestine: Report of 11 Cases and Review of Literature, Arch. Path. **27**:25, 1939.

3. Willis, R. A.: Pathology of Tumours, St. Louis, C. V. Mosby Company, 1948, p. 417.

4. Price, I.: Carcinoid Tumor of a Meckel's Diverticulum: Report of a Case, Brit. J. Surg. **23**:30, 1935.

5. Hertzog, A. T., and Carlson, L. A.: Carcinoid of Meckel's Diverticulum: Report of 2 Cases, Arch. Path. **20**:587, 1935.

6. Ashworth, C. T., and Wallace, S. A.: Unusual Locations of Carcinoid Tumors, Arch. Path. **32**:272, 1941.

7. Collins, D. C.; Collins, F. K., and Andrews, V. L.: Ulcerating Carcinoid Tumor of Meckel's Diverticulum: A Case Report, Am. J. Surg. **40**:454, 1938.

8. Pautler, E. E., and Scotti, T. M.: Carcinoid in Meckel's Diverticulum: Report of Case and Review of Literature Including Usual and Unusual Sites of Carcinoids, Gastroenterology **17**:88, 1951.

9. Stewart, M. J., and Taylor, A. L.: Carcinoid Tumor of Meckel's Diverticulum, J. Path. & Bact. **29**:135, 1926.

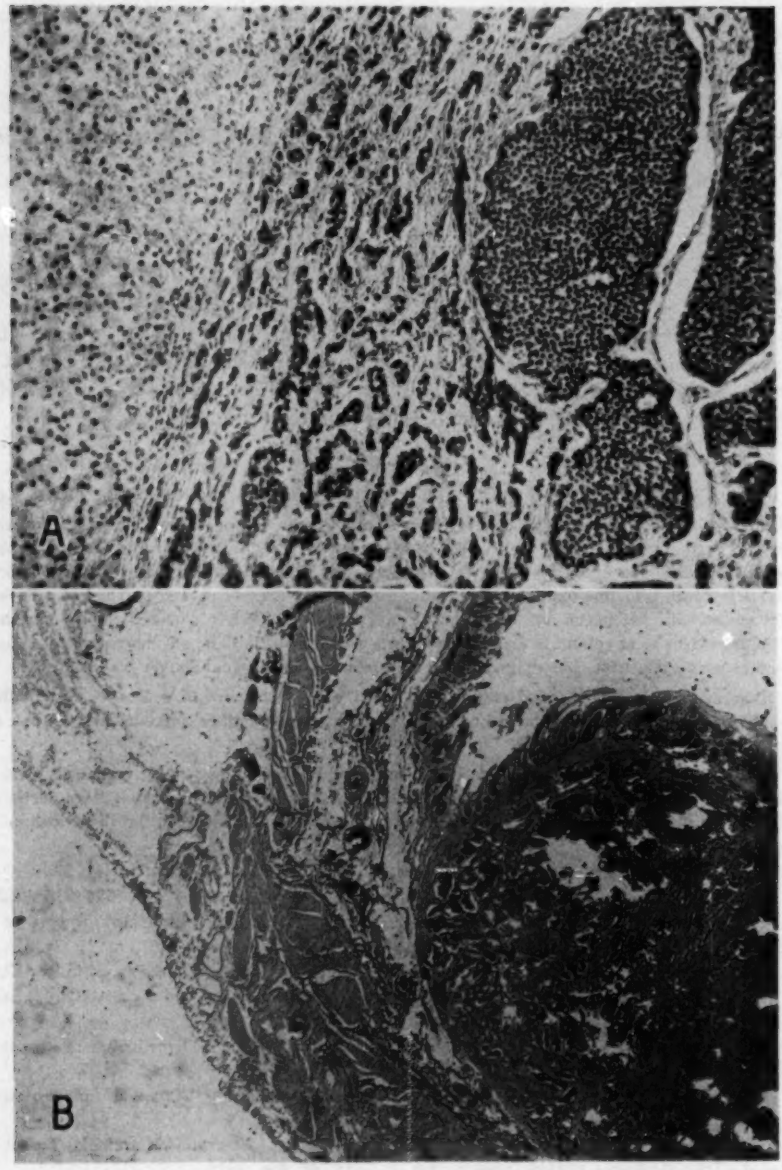


Fig. 1.—*A*, metastatic argentaffin carcinoma (carcinoid) in liver. Low-power view showing tumor on right and liver parenchyma on left. *B*, primary argentaffin carcinoma (carcinoid) in Meckel's diverticulum. Scanning view showing relation of the tumor in viscus.

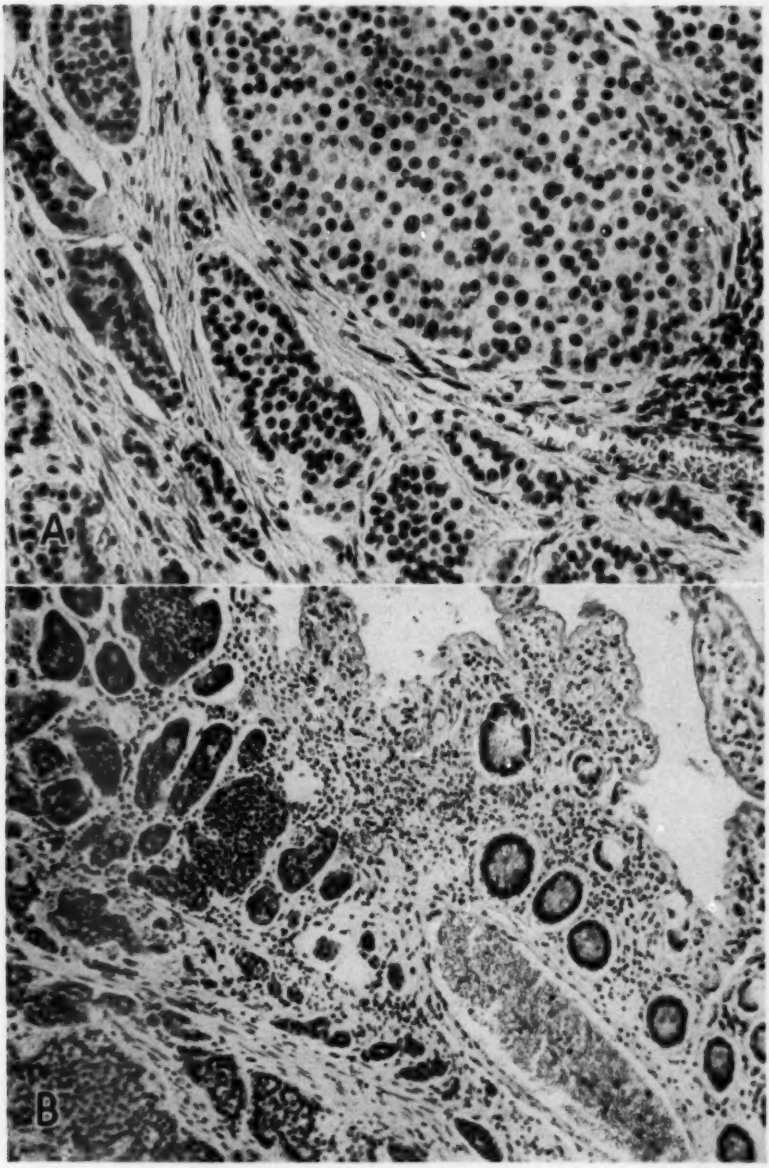


Fig. 2.—*A*, high-power view showing detailed histologic appearance of the primary tumor. *B*, low-power view of Meckel's diverticulum showing relationship between the tumor and mucosa. Note intimate gland-tumor relationship at upper left corner.

projected into, but did not obstruct, the lumen (Fig. 1*B*). Microscopically this tumor (Fig. 2*A*) was identical with that found in the liver. Although the largest portion of the tumor was present in the submucosa, at one point it had extended through the muscularis mucosae and appeared to arise from Kultschitsky cells at the base of a gland of Lieberkühn (Fig. 2*B*). Silver stains of the primary tumor and the metastasis were positive.

This case is reported as an example of argentaffin carcinoma (carcinoid) arising in a Meckel diverticulum and metastasizing to the liver. Microscopically it appears to support the theory of mucosal origin for these tumors from the Kultschitsky cells, with subsequent secondary involvement of the submucosa.[10]

10. Masson, P.: Carcinoids (Argentaffin-Cell Tumors) and Nerve Hyperplasia of the Appendicular Mucosa, Am. J. Path. **4**:181, 1928.

# Laboratory Methods and Technical Notes

## USE OF POTASSIUM CYANIDE IN THE GIEMSA STAIN

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Techniques for the Giemsa tissue stain may be found in Mallory,[1] Cowdry,[2] Lillie,[3] and Gatenby and Beams.[4] These appear to be modifications of Giemsa's [5] original method and were practiced in this laboratory to stain bacteria and to differentiate the structural elements of infected tissue. Difficulty was encountered in achieving these objectives. Step-by-step analysis of the procedures revealed that "de-Zenkerization" by dilute Lugol's solution impaired the capacity of the tissues to assume the Giemsa stain. Duplicate sections of these tissues, however, stained acceptably where the iodine-potassium iodide and sodium thiosulfate processes were omitted from the procedure.

The compromise might have been acceptable if it were not that the mercury salt crystals of the fixative (fluid containing bichloride of mercury) were found especially concentrated in tissues such as liver, spleen, and kidney. Further, while tissue sections were stained before "de-Zenkerization," there were irregularities in staining and in differentiation of the tissue structures.

These facts stimulated investigation of "de-Zenkerizing" by solutions other than dilute Lugol's and sodium thiosulfate. The observation of Gomori [6] that metals dissolve in dilute solutions of potassium cyanide is the basis of the work reported here.

### MATERIALS

The tissues were fixed in Zenker's [4] or in Technicon FU48 [7] solutions and preliminary preparation accomplished by the Autotechnicon [8] dehydration, clearing, and infiltration system. It was demonstrated that Technicon FU48 contained mercuric ion by the method described by Walton and Sorum.[9]

Potassium cyanide (0.05%) was prepared in Sørenson's phosphate buffer at pH 5.3 to 5.5. Buffer was prepared according to the directions of Cowdry.[2] The final pH of the solution was from 6.0 to 6.2. The buffer was used, since 0.05% potassium cyanide in water resulted in a solution of pH greater than 8.0. If the cyanide solution was too alkaline, the tissues did not stain well by Giemsa's stain. The solution was freshly prepared each time.

1. Mallory, F. B., and Wright, J. H.: Pathological Technique, Ed. 7, Philadelphia, W. B. Saunders Company, 1919.

2. Cowdry, E. V.: Laboratory Technique in Biology and Medicine, Ed. 2, Baltimore, Williams & Wilkins Company, 1948.

3. Lillie, R. D.: Histopathologic Technique, Philadelphia, The Blakiston Company, 1948.

4. Gatenby, J. B., and Beams, H. W., Editors: Microtomist's Vade-Mecum (Bolles Lee), Ed. 11, Philadelphia, The Blakiston Company, 1950.

5. Giemsa, G.: Über die Färbung von Feuchtpräparaten mit meiner Azur-Eosinmethode, Deutsche med. Wchnschr. **35**:1751-1752, 1909.

6. Gomori, G., in Methods in Medical Research, Visscher, M. B., Editor, Chicago, Yearbook Publishers, Inc., 1951, Vol. 4.

7. Technicon Formula No. FU48, Lot No. P566, 51, procured from Technicon Chemical Company, 215 East 149th St., New York 51, Feb. 26, 1951.

8. The Technicon Company, 215 East 149th Street, New York 51.

9. Walton, J. H., and Sorum, C. H.: Introduction to Qualitative Analysis, Menasha, Wis., George Banta Publishing Company, 1938.

Giemsa's stock solution,[10] prepared by the manufacturer according to the method of Lillie,[11] was used in all staining procedures.

The staining solution was prepared as follows: 100 ml. distilled water at pH 6.0 to 7.0, 3 ml. stock Giemsa stain added dropwise with constant stirring, 3 ml. absolute methyl alcohol.

### METHOD

Tissues were fixed in Zenker's or Technicon FU48 solutions, washed, and submitted to the Autotechnicon cycle of dehydration, clearing, and infiltration with paraffin. They were embedded in paraffin, blocked, and sections were cut at 7 μ. After fixation to slides by gelatin-potassium dichromate solution, prepared by the method of Cobe and Schaeuffle,[12] the sections were dried and treated as follows: xylene, 3 to 5 minutes; xylene, 3 to 5 minutes; absolute ethyl alcohol, 3 to 5 minutes; absolute ethyl alcohol, 3 to 5 minutes; 95% ethyl alcohol, 3 to 5 minutes; 95% ethyl alcohol, 3 to 5 minutes; 80% ethyl alcohol, 3 to 5 minutes; 70% ethyl alcohol, 3 to 5 minutes; distilled water (several changes), 3 to 5 minutes; 0.05% potassium cyanide in Sørenson's buffer at pH 5.3 to 5.5, 20 to 60 minutes or more depending upon the tissue; distilled water (several changes), 15 to 20 minutes; Giemsa stain (freshly prepared), 60 minutes; distilled water to wash off excess stain; 95% ethyl alcohol for differentiation, 5 to 10 minutes; absolute ethyl alcohol, 15 to 30 seconds; absolute ethyl alcohol, 15 to 30 seconds; xylene (to remove alcohol), 15 to 30 seconds; xylene (to clear), 3 to 5 minutes; xylene (to clear), 3 to 5 minutes; toluene, 3 to 5 minutes; mounted in clarite.

Exactly neutral water was not necessary in this method. Tissue sections were washed in distilled water of pH 6.0 to 7.0 where indicated. Tap water, since it is often alkaline, was not used.

The time required to clear a tissue section of mercury salt crystals with dilute potassium cyanide was found to vary with the thickness of the sections and the type of tissue. Sections of lung were apparently freed of crystals in from 15 to 20 minutes. Liver or kidney sections, on the other hand, required an hour or more in the solution. Although potassium cyanide was not as rapid a "de-Zenkerizing" agent as is Lugol's solution, the advantages of uniform and definite differentiation of the colors in the finished section outweighed the advantage of rapid action achieved by the latter solution.

Differentiation, made in 95% ethyl alcohol, was not a rapid process, thereby permitting closer control over the rate of reaction than that obtained, for instance, with dilute acetic acid. There was no need to rush the tissue sections into dehydration and clearing agents. This leisurely process allowed full and uniform development of the red blood cell color. At the same time the red and pink stains of muscle and connective tissue developed to greatest capacity. Simultaneously, excess of blue dye was eliminated.

In general, tissues stained as follows: red blood cells, orange to red; cytoplasm, blue, violet, or dark red, depending upon the cell or tissue; nuclei, blue to violet; smooth and striated muscle, red to pink or lavender; connective tissue, pink; cartilage, blue to purple; bacteria, blue to purple.

Ancillary studies indicated that timing of procedures, the nature of the dye compound, and the hydrogen ion concentration of the staining mixture were demonstrably important factors in analysis of the Giemsa stain procedure. But these lost some significance when compared with the fundamental effect on tissues of "de-Zenkerizing" by iodine-potassium iodide solutions.

Further studies of the stain where "de-Zenkerizing" was omitted showed that formalin added to the tissue fixative acted to accentuate the blue color in the Giemsa stain, as noted by Cowdry.[2] The effect of pH in the staining fluid was not as marked as one might expect from observations in Gatenby and Beams.[4] There was a tendency to predominance of red in acid solutions and of blue in alkaline solutions, but the differences in color values obtained over a pH range of from

10. Purchased from Gradwohl Laboratories, 3514 Lucas Avenue, St. Louis.

11. Lillie, R. D.: Some Experiments on the Romanovsky Staining of Blood Films, J. Lab. & Clin. Med. **28**:1872-1875, 1943.

12. Cobe, H. M., and Schaeuffle, A. A.: A Practical and Inexpensive Method for Fixation of Paraffin Sections to Slides, Am. J. Clin. Path., Tech. Sect. **10**:31-32, 1946.

6.0 to 8.0 were slight, and the stains were acceptable. Again, where "de-Zenkerizing" was eliminated, differentiation could be achieved in distilled water at pH 6.0 to 7.0 and without the use of 95% ethyl alcohol, although this reagent was used to hasten the process. This observation paralleled that of Lillie.[3]

SUMMARY

Tissues fixed in Zenker's or Technicon FU48 solutions were found difficult to stain by several modifications of the Giemsa stain. It was determined that the difficulty arose through treatment of the tissue sections with dilute Lugol's solution followed by one of sodium thiosulfate to remove excess iodine. Insoluble mercury compounds were removed from the sections by this treatment but with a simultaneous loss in the capacity of the tissue elements to assume delicate differential stains in a uniform manner.

Acceptable Giemsa staining resulted if the conventional "de-Zenkerizing" method was omitted after tissue sections were brought to water. Upon completion of the stain, however, the undesirable crystals remained.

An alternative method of removing the crystals was presented in the use of 0.05% potassium cyanide in Sørenson's phosphate buffer at pH 5.3 to 5.5. The crystals were found to be soluble in this reagent in 20 minutes to 1 hour or more, depending upon the tissue.

# NEW METHOD FOR STAINING MYCOBACTERIA IN TISSUE SECTIONS

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The identification of Mycobacterium tuberculosis and Myco. leprae in tissue sections is dependent upon the amount of contrast obtainable between the organisms and the surrounding material. Since Myco. tuberculosis is resistant both to staining and to destaining, one method has been to stain with a phenol-containing dye solution (which overcomes the staining resistance), partially destain non-acid-fast material with acid-alcohol, and then stain the background with a contrasting aqueous dye. This method relies on the assumption that the acid-fast organisms will not take the counterstain. It has recently been reemphasized,[1] however, that many acid-fast bacteria, such as Myco. leprae, although resistant to acid-alcohol differentiation, did not possess the definite staining resistance seen in Myco. tuberculosis. Further, staining resistance was easily abolished (as by crushing) from those cells where it was normally found. It was sometimes, though not always, true that the acid-fastness of non-stain-resistant types of mycobacteria was masked when they were covered by counterstain. Preliminary study showed that the same phenomenon could be seen in tissue lesions containing Myco. tuberculosis. In almost every tubercle there were a few organisms which were acid-fast but which were not seen after counterstaining and so were presumptively covered by the second dye.

Since the counterstain then has theoretical and practical disadvantages, it would appear more desirable to obtain the contrast between acid-fast bacilli and background in another manner. The simplest way would be to increase the amount of dye removed from the background tissue by an acid-alcohol to which the stained mycobacteria were resistant. Without a counterstain however, the fuchsins (the standard dyes for demonstrating acid-fast organisms) stained the background much too heavily. After differentiation with hydrochloric or acetic acid-alcohols there was usually so little contrast between mycobacteria and tissue elements that only massed bacilli could be seen and identified. There were several basic dyes, notably crystal violet, which left no residual background stain after differentiation, but in every instance these proved too weak: Individual isolated mycobacteria were too poorly stained to be seen. Auramine O [2] was satisfactory as to residual stain and brilliance, but its use on tissues as a fluorescent dye led to confusion between stained bacteria and autofluorescent tissue elements.

In other words, of the dyes presently available, only the fuchsins (especially new fuchsin, C. I. 678 [3]) stained the mycobacteria as brilliantly as was necessary if the few in a given tissue lesion were to be seen. Since differentiation with the usual hydrochloric, nitric, sulfuric, and acetic acid-alcohols appeared unsatisfactory, other types of acid were tried. Sulfurous acid as a reducing acid and bleaching agent had been tried [3] with little success. It and other reducing acids were retested but in alcohol rather than in aqueous solutions. All rapidly removed most of the residual background dye, and two (oxalic and formic acids) did this while removing the

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This study was aided by a grant from the National Cancer Institute, United States Public Health Service.

Dr. Berg is a Postdoctorate Fellow, National Cancer Institute. He is now at the Department of Pathology, Memorial Center.

1. Berg, J. W.: The Dual Nature of Acid-Fastness, to be published.

2. Richards, O. W., and Miller, D. K.: An Efficient Method for the Identification of M. Tuberculosis with a Simple Fluorescence Microscope, Am. J. Clin. Path. Tech. Supp. **5**:1-8, 1941.

3. Fite, G. L.: The Staining of Acid-Fast Bacilli in Paraffin Sections, Am. J. Path. **14**:491-507, 1938.

color more slowly from acid-fast cells. Thus definite and useful differentiation was achieved without a counterstain, and the sensitivity of the stain as a diagnostic procedure was thereby increased.

## METHODS AND RESULTS

Of the reducing acids tested, sulfurous acid proved too strong, even in alcohol. At a strength of 1% it removed the color from the mycobacteria at about the same rate it decolorized the background. Oxalic and formic acid-alcohols in concentrations of 1 to 10% acid acted more slowly. There was a definite, though not wide, margin of safety (four minutes approximately) between decolorizing of the background and destaining of the cells. (This was in contrast to a 15-minute margin for acetic acid-alcohol acting on Myco. leprae.) The two acids acted much the same. Formic acid was chosen for further study only because it was more convenient and more rapid to prepare the acid-alcohols from the liquid acid rather than from the solid oxalic acid.

Studies on the optimal concentrations of acid and alcohol led to the observation that the amount of undesirable residual background stain was almost as much affected by the type of tissue fixation as by the differentiating agent. Eighty per cent alcohol proved to be the fixative of choice (see the results below), but the method gave improved results, even with tissues fixed in formaldehyde or in Helly's solution, the routine fixatives in this laboratory.

The dye solution, alkaline carbol-fuchsin, was chosen to give rapid, as well as intense, staining. A stock solution was prepared from a 5% solution of new fuchsin (C. I. 678) in absolute alcohol. This was filtered, and to it was added 25 ml. of melted phenol for each 100 ml. of alcohol-dye solution. The mixture proved stable indefinitely. At the time of use, one part of the stock solution was mixed with four parts of an aqueous buffer, pH 7.8 (for the M/15 Sorenson phosphate buffer, 8.56 gm. anhydrous $Na_2HPO_4$ and 0.78 gm. $KH_2PO_4$ per liter of water).

The staining procedure was as follows:

1. Embed the fixed tissue in paraffin, section, mount, and deparaffinize in the usual manner.
2. Stain in freshly prepared alkaline carbol-fuchsin for five minutes at room temperature.
3. Wash thoroughly in two changes of 95% alcohol for a total time of one minute.
4. Differentiate for three minutes in formic acid-alcohol: (*a*) 5% formic acid in absolute alcohol for tissues fixed in 80% alcohol and 4% formaldehyde; (*b*) 1% formic acid in absolute alcohol for tissues fixed in Helly's solution.
5. Wash in three changes of absolute alcohol, one minute each.
6. Clear in xylene and mount.

*Results.*—Alcohol-Fixed Tissues: Mycobacteria were brilliant red, nuclear chromatin pale red; the rest of the tissue elements were not stained.

Formaldehyde-Fixed Tissue: Mycobacteria were brilliant red, nuclei bright red to pink, background material pink.

Helly's-Fixed Tissues: Mycobacteria were brilliant red, nuclei pale red to pale pink, background pale pink.

## COMMENT

The greater amount of residual background seen in tissues which had been fixed in formaldehyde was apparently due to inadequate washing, resulting in turn in retention of formaldehyde in the tissues and a reaction between fuchsin and formaldehyde similar to that between the dye and other aldehydes. By contrast, tissues fixed in Helly's solution were exposed to the same 4% formaldehyde but were subsequently washed for 24 hours. They showed much less background stain. The important disadvantage of Helly's solution as a fixative arises from the fact that potassium dichromate is a specific inhibitor of acid-fastness.[4] Even the short exposure of the cells to the dichromate in Helly's solution made them more sensitive to differentiation.

In each case, then, the differentiating agent was matched to the fixative so as to provide the greatest contrast between bacilli and their background. In general, there was less contrast when 95% alcohol was substituted for absolute alcohol in the differentiating mixture. While in all instances nuclear chromatin tended to stain almost as heavily as the acid-fast organisms, there

4. Berg, J. W.: Acid-Fastness as a Histochemical Reaction, J. Histochem. & Cytochem., to be published.

was almost no confusion between the two materials because of their completely different sizes and configurations. On the other hand, there had been a great deal of bacillus-sized debris left acid-fast after HCl-alcohol differentiation. This was no longer strongly stained, and its absence greatly speeded the search for bacteria.

Rapid staining at room temperature was first achieved by Putt,[5] who used a wash of saturated lithium carbonate after staining. Further study showed that the alkalinity (pH 10) of the solution was almost surely the factor which speeded the staining. The postulated mordanting action of the solution was manifested by increased residual background stain. The use of alkaline carbol-fuchsin has eliminated the undesirable mordanting, but the dye solution, in contrast to standard carbol fuchsins, proved to be unstable, and it was necessary to prepare a fresh staining solution each day. Hence under some laboratory conditions, Putt's method of obtaining alkaline catalysis may prove the more practical.

The method as given here permitted more rapid and more accurate evaluation of tissue lesions suspected to contain mycobacteria. The number of false negative readings in a series of surgical and autopsy material was decreased by 10% when the tissues, previously evaluated by other techniques, were studied by the present method. Still almost one-third of all sections from diagnosed cases of tuberculosis were negative for acid-fast bacilli by all methods of staining. The results varied from the fulminating cases, in which all techniques showed organisms in all sections, to those cases which were inactive and in which no organisms were found by any method. The intermediate group, in which perhaps 1 to 10 bacilli were present in each tubercle, was where the new method proved most useful. Still the results were not all that would be desired, and two possible explanations suggest themselves. First, where organisms were not found in active lesions, it was invariably true that the patient had been given intensive courses of antibiotics. Hence, it may well have been that there were few, if any, surviving organisms. On the other hand, it must be admitted that the method of staining reported here, like others, is not theoretically perfect. It was noted in previous work [1] that Myco. tuberculosis deprived of its "structural" acid-fastness was not strongly stained, even after acetic acid-alcohol differentiation. Formic acid-alcohol is a stronger differentiating agent, and completely destained organisms from old colonies of Myco. ranae (grown on Patrograni's media) which had been acid-fast with weaker differentiation. Thus the dilemma is unsolved. It remains evident that if the differentiating agent is strong enough to decolorize the background, it is also strong enough to decolorize some acid-fast bacteria. Therefore, if a basic dye as selective as crystal violet but as intense after differentiation as fuchsin could be found, it is highly probable that the sensitivity of the acid-fast diagnostic test would be increased over what we have been able to achieve so far.

## SUMMARY AND CONCLUSIONS

Since the use of a counterstain reduces the sensitivity of the acid-fast reaction, it was considered desirable to obtain high contrast between acid-fast bacilli and non-acid-fast background tissue in some other manner. This was accomplished by using a reducing acid, formic acid, in the differentiating acid-alcohol solution. Rapid staining at room temperature was achieved by making the carbol-fuchsin solution alkaline. The results were affected by the type of fixation; alcohol-fixed tissue gave better results than did tissues fixed in formalin or Helly's solution.

Help and encouragement were given by Dr. H. Bunting, in whose laboratory this study was done.

5. Putt, F. A.: A Modified Ziehl-Neelsen Method for the Demonstration of Leprosy Bacilli and Other Acid-Fast Organisms, Am. J. Clin. Path. **21**:92-95, 1951.

# News and Comment

**Cancer Seminars.**—The Second Annual Cancer Seminar of the Arizona Division of the American Cancer Society will be held on Jan. 14, 15, and 16, 1954, at Paradise Inn in Phoenix, Ariz.

The program is planned as a series of panel discussions on (1) Recent Advances in Cancer Therapy, (2) Neoplasms of the Central Nervous System, (3) Carcinoma of the Cervix, (4) Carcinoma of the Breast, (5) Exfoliative Cytology, (6) Surgical Methods for Relief of Intractable Pain, (7) Carcinoma of the Lung, (8) Trends in Cancer Research, (9) Leukemia and Lymphomas, and (10) Tumors of Childhood.

Participants in the panel discussions are Paul C. Aebersold, Ph.D.; Percival Bailey, M.D.; O. A. Brines, M.D.; C. S. Cameron, M.D.; Anthony Curreri, M.D.; Michael DeBakey, M.D.; L. H. Garland, M.D.; E. S. Gurdjian, M.D.; Joe V. Meigs, M.D.; E. P. Pendergrass, M.D.; Orvar Swenson, M.D., and D. A. Wood, M.D.

Reservations may be obtained by writing to the Arizona Division, American Cancer Society, 1429 North First Street, Phoenix, Ariz.

The Third Annual Slide Seminar on Unusual Tumors will be held in the Nurses' Auditorium of the Presbyterian Hospital, Newark, N. J., at 2 p. m. on Dec. 12, 1953.

The seminar is sponsored by the New Jersey Society of Clinical Pathologists. Drs. Fred Stewart and Frank Foote Jr., of Memorial Center, New York, will serve as moderators.

**Annual Meetings.**—The Wisconsin Society of Pathologists held its annual meeting in Milwaukee on Oct. 7, 1953. The following officers were elected for the ensuing year: president, Dr. E. A. Birge, Milwaukee; vice-president, Dr. M. B. Llewellyn, Janesville; secretary-treasurer and councilor to The American Society of Clinical Pathologists, Dr. Robert S. Haukohl, Milwaukee; delegate to State Medical Society, Dr. Gorton Ritchie, Milwaukee; alternate delegate, Dr. Robert S. Haukohl, Milwaukee; board of censors, Dr. S. B. Pessin, Milwaukee; Dr. Etheldred L. Schafer, Madison, and Dr. Walter H. Jaeschke, Madison.

The scientific program consisted of the following topics: "The Medicolegal Aspects of Sudden Death," L. J. Van Hecke, M.D., Associate Professor of Pathology, Marquette University; "The Pathology of Hemorrhagic Fever," Brig. Gen. Elbert DeCoursey, M.D., Director, Armed Forces Institute of Pathology, Washington, D. C.; "Tumors in Childhood," Gorton Ritchie, M.D., Milwaukee. Brig. Ben. Elbert DeCoursey presented a paper on "Tissue Reaction to Ionizing Radiation" at the round table luncheon.

**Pathologists Honored.**—Edith Potter, M.D., Associate Professor of Pathology, University of Chicago, and Pathologist to the University of Chicago Lying-In Hospital, was awarded an honorary degree by the University of Brazil on Oct. 1, 1953. Dr. Potter received this award at the dedication of the University's new hospital for children under 2 years of age.

James W. Jobling, M.D., and Alwin M. Pappenheimer, M.D., each of whom is an Emeritus Professor of Pathology at Columbia University, received Distinguished Service Awards in conjunction with the 25th anniversary of Columbia-Presbyterian Center on Oct. 12 and 13, 1953.

James E. Ash, M.D., is the recipient of the Ward Burdick Award for 1953. This citation was presented to Colonel Ash during the annual meeting of the American Society of Clinical Pathologists.

Leonard W. Larson, M.D., received a Scroll of Merit from the American Society of Clinical Pathologists in recognition of his distinguished contribution to medicine and to pathology.

**Armed Forces Institute of Pathology.**—The ceremony attending the laying of the cornerstone of the new Armed Forces Institute of Pathology was held at 10 o'clock on Tuesday, Oct. 20, 1953, at the Walter Reed Army Medical Center, Washington, D. C.

The trowel used in this ceremony is made of parts of an early microscope from the Medical Museum collection.

**Appointments.**—Robert A. Moore, M.D., Dean of the College of Medicine and Professor of Pathology, Washington University, St. Louis, has been appointed Vice Chancellor of the Health-Science Division of the University of Pittsburgh. Dr. Moore has served as professor of pathology at Washington University since 1939. He will relinquish his present position at the close of the present academic year.

# Books

**Chemical Induction of Cancer.** By George Wolf, B.S., Ph.D., Assistant Professor, University of Illinois; Formerly Research Fellow, McArdle Memorial Laboratory, University of Wisconsin and Harvard University, and Research Associate at The Chester Beatty Research Institute, The Royal Cancer Hospital, London. Price, $3.50. Pp. 250, with 5 illustrations. Harvard University Press, Cambridge 38, Mass., 1952.

This is a good survey of the chemical carcinogens and their role in experimental cancer research. Chemical structure is related to carcinogenic activity for the polycyclic aromatic hydrocarbons. Their use by the biologist, their possible mode of action, the possibility of their production in the organism, and their metabolism are successively treated. The carcinogenic azo compounds which induce hepatic cancer, the aromatic amines (e.g., 2-aminofluorene), and various "miscellaneous" carcinogens (e.g., arsenic, halogenated hydrocarbons, urethane) are considered. Finally, the possibilities of tumor chemotherapy are discussed.

The book not only serves as a good orientation for those who are casually interested in the field, but also is sufficiently documented by references to be useful to the research worker. It is not merely a report of what has been done. The presentation is thought-provoking, and the book is to be recommended as a special reading assignment for graduate and medical students.

**Pathology of the Heart.** Edited by S. E. Gould, M.D., D.Sc., Clinical Professor of Pathology, Wayne University College of Medicine, Detroit; Pathologist, Wayne County General Hospital, Eloise, Mich.; Consultant in Pathology, Veterans Administration Hospital, Dearborn, Mich., and Editor, *American Journal of Clinical Pathology*. Price, $25.00. Pp. 1023, with numerous illustrations and tables. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1953.

American pathologists have felt for a long time the need for comprehensive work in the various fields of pathology which, above and beyond the coverage offered by the usual textbooks, can be used as a source of complete and authoritative information. Several such books, many of them excellent, have appeared in recent years. This volume fulfills such a need for pathology of the heart, and, as Dr. Karsner states in the preface, it is a major contribution attesting to growth and maturity of American pathology.

The book, published under the editorship of S. E. Gould, is the result of a cooperative effort by a group of outstanding pathologists well known for their competence in the field of cardiovascular diseases. Congenital malformations of the heart and great vessels are lucidly and thoroughly discussed in a long chapter (238 pages) contributed by J. E. Edwards. Degenerative diseases are described by W. B. Wartman and W. T. Hill and vascular lesions of the heart by S. E. Gould. Rheumatic and nonrheumatic inflammatory diseases are discussed in two excellent chapters, respectively, by A. H. Baggenstoss and O. Saphir. These are followed by sections on parasitic diseases (W. A. Stryker), injuries by physical violence (A. R. Moritz), and neoplasms (O. Saphir). The central portion of the volume is preceded by a short historical introduction (E. B. Krumbhaar) and by extensive chapters on development and structure (B. M. Patten and A. Barry) and on physiology of the heart (H. D. Green). A discussion of clinicopathologic correlation is contributed by G. B. Myers. A useful appendix on gross examination, weights, and measurements of the heart and injection of coronary arteries (O. Saphir) is included. There are more than 250 well-chosen and well-reproduced illustrations. An extensive bibliography follows each chaper. Well-prepared subject and author indices conclude the volume.

This undoubtedly is the most complete, authoritative, and up-to-date book of its kind in the English literature. The style is lucid and homogeneous throughout. Without being encyclopedic, coverage of most subjects is generally complete and obviously the result of much thought and experience. There is, however, a certain lack of balance between the thorough introductory chapters and the rest of the book. In particular, degenerative, nutritional, and metabolic disorders are discussed rather briefly. Chemical features of the myocardium in normal and abnormal conditions are not discussed at all. Correlation between lesions and symptoms and physiopatho-

logical remarks would have been more effective if completely discussed with each disease rather than in separate chapters. Inclusion of a few color reproductions of key pathologic entities would have been desirable. Only occasional misprints were noted, but in the copy received by this reviewer there is a serious error of pagination between pages 570 and 606. These imperfections do not detract from the value of the volume and would be easily corrected in a second edition.

The editor and the authors should be congratulated on a superb contribution. The book is a "must" for every pathologist and cardiologist and can be usefully consulted by every physician.

**Multiple Myeloma.** By I. Snapper, M.D., Director of Medical Education, Cook County Hospital, Chicago; Formerly, Director of Medical Education, Mount Sinai Hospital, New York; Louis B. Turner, M.D., Research Assistant in Medicine, Mount Sinai Hospital, New York, and Howard L. Moscovitz, M.D., Resident Second Medical Service, Mount Sinai Hospital, New York. Price, $6.75. Pp. 168, with 43 illustrations. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1953.

This small volume draws together in its thirteen chapters most of the existing information on the nature, findings, course, and therapy of multiple myeloma.

Its content is indicated by the chapter headings, as follows: (1) The Myeloma Cell, (2) Incidence and Survival, (3) The Peripheral Blood Picture, (4) Clinical Manifestations, (5) Roentgenologic Changes in the Bones, (6) Metabolic Abnormalities in Multiple Myeloma, (7) Solitary Myeloma, (8) Multiple Plasmocytomas, (9) Extramedullary Plasmocytomas, (10) Pathology, (11) Differential Diagnosis, (12) Treatment of Myeloma, and (13) Summary.

The volume is illustrated adequately with reproductions of roentgenograms, photographs, photomicrographs, and graphs. A list of two hundred seventeen key references is appended, and there is a well-constructed index.

The book can be recommended to physicians and students who are seeking information concerning any aspect of the mysterious disorder known as multiple myeloma.

**Clinical Management of Behavior Disorders in Children.** By Harry Bakwin, M.D., Professor of Clinical Pediatrics, New York University; Visiting Physician, Bellevue Hospital; Attending Pediatrician, University Hospital, and Ruth Morris Bakwin, M.D., Associate Professor of Clinical Pediatrics, New York University; Associate Visiting Physician, Bellevue Hospital; Director of Pediatrics, New York Infirmary. Price, $10.00. Pp. 495, with 14 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, and 7 Grape St., Shaffesbury Ave., London, W.C. 2, 1953.

**Bone Tumors.** By Louis Lichtenstein, M.D., Senior Pathologist, General Medical and Surgical Hospital, Veterans Administration Center, Los Angeles; Formerly Associate Pathologist, Hospital for Joint Diseases, New York; Consultant in Bone Tumors, Tumor Registry of the California Medical Association Cancer Commission; Sometime Lecturer in Bone Pathology, Medical Extension, University of California at Los Angeles. Price, $10.50. Pp. 315, with 155 illustrations. C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1952.

This book, as is noted in the preface, is an outgrowth of a long series of published studies of tumors of bone. During the period 1938-1948 many of the author's investigations were conducted in collaboration with Henry L. Jaffe, M.D., at the Hospital for Joint Disease, in New York.

The present volume draws together in concise form the information derived from these studies of neoplasms and other disorders of bone. Also, it records observations which the author has made in recent years at the Veterans Administration Center in Los Angeles.

The first two chapters are assigned respectively to pertinent general remarks and classification. Each succeeding chapter, through Chapter 21, is devoted to one form of skeletal neoplasm. Chapter 22 is employed to comment on carcinoma metastatic to the skeleton, and an appendix is used for brief mention of a few non-neoplastic lesions (fibrous dysplasia, eosinophilic granuloma, Paget's disease, etc.) which may simulate true tumors.

The neoplasms to which individual chapters are assigned include osteocartilagenous exostosis, solitary enchondroma, benign chondroblastoma, chondromyxoid fibroma, osteoid osteoma, osteogenic fibroma, nonosteogenic fibroma, giant-cell tumor, tumors of vascular origin, tumors of nerve origin, chondrosarcoma, osteogenic sarcoma, fibrosarcoma, Ewing's sarcoma, multiple myeloma, tumors of hematopoietic origin (including leukemia, lymphosarcoma, and Hodgkin's disease), liposarcoma, chordoma, and adamantinoma of limb bones.

In the respective chapters the author describes the clinical, radiologic, and pathologic features of a given neoplasm. He then devotes a section to treatment and prognosis, and this is followed by a concise summary. A useful and perhaps adequate list of references concludes each chapter.

The illustrations provided in 155 cuts are well chosen and depict the roentgenologic and the gross and microscopic pathologic features of each entity.

Some readers may be disappointed because of the brevity of the treatment of some entities. However, anyone who has reason to be concerned with the diagnosis or management of neoplastic disorders of the skeleton will find it profitable to have this volume available for reference.

This book is recommended especially to pathologists, radiologists, and surgeons. It will be particularly valuable to residents in training in these specialties of medicine.

**The Sulphonamides and Antibiotics in Man and Animals.** By J. Stewart Lawrence, M.D. (Ed.), M.R.C.P., Lecturer, The Department of Occupational Health; The University of Manchester Out-Patient Medical Officer, The Salford Royal Hospital, and John Francis, M.Sc., M.R.C.V.S., Professor of Preventive Medicine, University of Queensland Veterinary School, Brisbane, Australia; with the Assistance of Arnold Sorsby, M.D., F.R.C.S., Research Professor in Ophthalmology, The Royal College of Surgeons and Royal Eye Hospital Surgeon, Royal Eye Hospital, London, and Philip G. Scott, F.R.C.S., Consultant Surgeon, Eye, Nose, and Throat Department, Royal Devon and Exeter Hospital, Exeter, England. Second Edition. Price, 42 shillings. Pp. 482, with 39 illustrations. H. K. Lewis & Co., Ltd., 136 Gower St., London, W.C.1, 1953.

**The 1952 Year Book of Pathology and Clinical Pathology, 1952.** Pathology edited by Howard T. Karsner, M.D., LL.D., Medical Research Advisor to the Surgeon General, United States Navy; Clinical Pathology edited by Arthur Hawley Sanford, M.A., M.D., Emeritus Professor of Clinical Pathology, Mayo Foundation; Emeritus Consultant, Division of Clinical Laboratories, Mayo Clinic; Director of Laboratories, Rochester State Hospital. Price, $5.50. Pp. 400, with 164 illustrations. Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1953.

This new edition of the Year Book furnishes once again a ready compilation of the significant and important work in pathology for the year 1952. The busy pathologist is indeed grateful for abstracts of current studies. Dr. Karsner's critical comments in the field of anatomic pathology are as usual very welcome. This issue of the Year Book does not have any of the longer critical reviews featured previously but in its brief abstracts continues to furnish a needed service.

**The Riddle of Cancer.** By Charles Oberling, M.D., translated by William H. Woglom, M.D. Revised edition. Price, $5.00. Pp. 238. Yale University Press, 143 Elm St., New Haven 7, Conn., 1952.

This is an excellent popular treatise of interest to a very wide audience, both scientific and lay. The various facets of cancer research (viruses, hormones, diet, genetics, carcinogens) are considered and woven into the riddle. A vast amount of scientific material is presented both accurately and dramatically. To begin reading the book guarantees finishing it, since the breathless style is such that it reads as a novel. The original was published in 1944, and this revision brings it up-to-date.

**Sexual Behavior in the Human Female.** By Alfred C. Kinsey, Wardell B. Pomeroy, Clyde E. Martin, Paul H. Gebhard, Research Associates, and others on the staff of the Institute for Sex Research, Indiana University. Price, $8.00. Pp. 842, with 151 charts, 179 tables, 4 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1953.

**Twenty-Five Years of Sex Research: History of the National Research Council Committee for Research in Problems of Sex, 1922-1947.** By Sophie D. Aberle, Member of the National Science Board of the National Science Foundation, and George W. Corner, Carnegie Institute of Washington. Price, $4. Pp. 248. W. B. Saunders Company, 518 W. Washington Sq., Philadelphia 5, 1953.

**The Theory of Wholes in Chemistry and Its Bearing on the Nature of Biological Catalysis: An Essay in Theoretical Chemistry.** By J. Vine, M.B., Ch.B. Price, $3. Pp. 97. Newman Wolsey Limited, Holborn Place, W.C. 1, London, 1953.

**Enzymatic Concept of Anaphylaxis and Allergy and the Role of Eosinophils in Anaphylactic Reactions Related to Hormonal Alterations.** By Z. Z. Godlowski, M.D., (Cracow), Ph.D. (Edin.), M.R.C.P. (Edin.), Research Fellow of the Carnegie Trust, Edinburgh; Physician to Ballochmyle Hospital, Ayrshire; Acting Professor of Physio-Pathology, and Lecturer in Clinical Medicine at the late Polish School of Medicine, Edinburgh University. Price, $3.50. Pp. 120, with 17 illustrations and 8 tables. E. & S. Livingstone, 16 and 17 Teviot Place, Edinburgh 1, 1953.

**Modern Clinical Psychiatry.**—By Arthur P. Noyes, M.D., Superintendent, Norristown State Hospital, Norristown, Pa.; Associate Professor of Psychiatry, Graduate School of Medicine, University of Pennsylvania. Fourth Edition. Price, $7.00. Pp. 609. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia & London, 5 and 7 Grape St., Shaffesbury Ave., W.C.2, 1953.

**The Breast in Roentgen Diagnosis.** By Raul A. Leborgne, M.D., Head of the Radiologic Department of the Instituto de Radiologia y Centro de Lucha Contra el Cancer, Hospital Pereira Rossell, Montevideo, Uruguay. English translation by Lucy Crocker de Leborgne. Price, not given. Pp. 194, with 307 illustrations. Impresora Uruguaya, Juncal 1511 Montevideo, Uruguay, 1953.

**Current Research on Vitamins in Trophology. Proceedings of the Scientific Sessions of the Eighth Annual Meeting, The National Vitamin Foundation, Incorporated, New York, March 4, 1953; Nutrition Symposium Series No. 7.** By W. F. Alexander, S. Corbo, W. J. Darby, Mei Yu Dju, G. Frontali, R. Funaro, M. A. Guzman, M. K. Horwitt, N. Jolliffe, B. M. Kagan, G. Lanciano, E. W. McHenry, G. Maggioni, K. E. Mason, N. S. Scrimshaw, W. T. Tompkins, and Dorothy G. Wiehl. Price, $1.50. Pp. 157. The National Vitamin Foundation, Inc., 15 E. 58th St., New York 22, 1953.

**A Text-book of Pathology: An Introduction to Medicine.** By William Boyd, M.D., Dipl. in Psychiat. M.R.C.P. (Edin.), F.R.C.P. (Lond.), F.R.C.S. (Canada), LL.D. (Sask.), D.Sc. (Man.), M.D. (Oslo), F.R.S. (Canada); Professor of Pathology, University of British Columbia, Vancouver, B. C., Canada. Sixth Edition. Price, $12.50. Pp. 1024, with 570 illustrations and 32 colored plates. Lea & Febiger, 600 S. Washington Sq., Philadelphia 6, 1953.

Dr. Boyd's "Text-Book of Pathology" is, in the reviewer's opinion, one of the most brilliantly written and readable textbooks in the English language. A page-by-page comparison of the old fifth edition and the present sixth edition reveals the artistry with which an inserted phrase or an additional sentence adds polish, clarity, and flair to the already excellent prose. The new edition is some thirty pages shorter than the previous one; yet it contains seventy more black and white illustrations and an entirely new and excellent chapter on skin diseases. Throughout the book, many newer observations and concepts have been incorporated. Pathologists will not agree with some of his statements, but any text by a single author will always meet the same criticism.

The subject has grown so complex that no single text can adequately cover the field, and any attempt at encyclopedic coverage is serious error. What is intended for second-year medical students primarily should be of quite different character from that designed for the pathology

resident. Textbooks for medical students should get smaller, instead of larger. This edition, shorter than the preceding one, is a step in the right direction. But, in the reviewer's opinion, an established author, like Boyd, could render an enormous service to pathology, and to pedagogy, if he boldly came out with this doctrine: that we are now trying to teach our medical students too many facts and make too great a fetish of "the latest discoveries"; that we can serve education best by teaching a smaller number of facts, but teach them in greater detail and clarity; that encyclopedic coverage should be left for yearbooks, monographs, and large reference works. We hope that the next edition will be still smaller.

In reference to the present work there are three specific criticisms: The classifications of carcinoma seem weak and illogical, especially carcinoma of the breast. The black and white illustrations are in general very good, but many can still be improved. And the colored plates, paying great tribute to the trichrome stains, and hallowed by many editions, nevertheless seem to be a waste of money.

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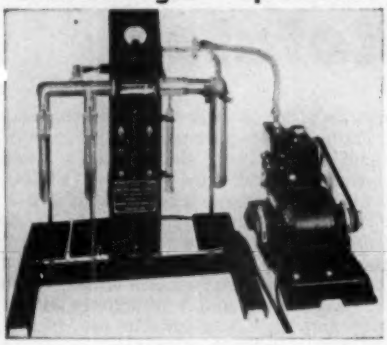

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BIBLIOGRAPHY

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